Intraspecific and interspecific social variation in the sweat bee *Lasioglossum malachurum* and other members of the subgenus *Evylaeus*

By

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A thesis
submitted to the Department of Biological Sciences
in partial fulfilment of the requirements for the
degree of

Master of Science

February 2002

Brock University

St. Catharines, Ontario

ABSTRACT

Sweat bees exhibit a range of social behaviours, from solitary nesting, in which no workers are produced, to strong eusociality, in which workers exhibit a high degree of altruism, behaviour that is measured by the degree of personal reproductive sacrifice. Field studies were carried out for seven weeks during May-June 2000 in southern Greece in order to investigate intraspecific social variation, and test the hypothesis of a north-south cline of decreasing eusociality in the obligately eusocial sweat bee *L. (E.) malachurum*. A comparative study, using principal components analysis, was performed to determine if patterns of intraspecific social variation in *L. malachurum* reflect the patterns of social variation within the subgenus, *Evylaeus*, as a whole.

The results of the field study reveal that, in Greece, two worker broods were produced followed by a third brood consisting of gynes, males and some workers, indicating that there was an overlap in worker and gyne production. There was strong caste distinction between queens and workers. Workers actively foraged and participated in nest construction as most workers (58%, n=303) had a high degree of mandibular wear. Workers did not participate in the oviposition of Brood 3 gynes since only 0.7% (n=278) of workers were mated. Furthermore, queen survival until the end of Brood 3 and a substantial size differential of 10.6% between queens and workers suggested that queen domination over worker behaviour during the early to mid-part of the colony cycle was plausible. Male production in Brood 3 by some workers was likely, since the timing of worker ovarian development corresponded with the timing of male production. These findings suggest that workers of the first two broods were primarily altruistic, but some (28%) Brood 1 (9%) and Brood 2 (19%) workers produced males, indicating that the degree of altruistic behaviour declined during the lifetime of the colony. In comparison with other *L. malachurum* populations in Europe, the Greek population of *L. malachurum* had a weaker social level as a result of the higher proportion of workers potentially involved in male production, thus

supporting the hypothesis of a southerly cline of decreasing eusociality. Furthermore, intraspecific variation in social level across Europe appears to be due to longer breeding seasons in more southerly locations that would promote the production of larger colonies and provide opportunities for workers to evade queen control.

The comparative study using principal components analysis on 20 solitary (of the subgenera *Evylaeus* and *Lasioglossum*), eusocial and socially polymorphic *Evylaeus* species and populations reveals that six traits are closely associated with stronger eusociality in *Evylaeus*. These traits are: (1) a reduction in the proportion of males in the early brood(s); (2) a reduction in the proportion of females that mate; (3) an increase in the mean number of first brood workers; (4) a reduction in the proportion of females with developed ovaries; (5) an increase in size dimorphism between castes, and (6) nest guarding. These are traits that most significantly define principal component one and therefore distinguish social type as indicated by a clear separation of the eusocial and the solitary populations, with a socially polymorphic species falling in between. Furthermore, most of these traits are under foundress control and may suggest that the evolutionary loss or gain of eusociality is based on selection pressures on a founding female. Colony size and female ovarian development are common factors distinguishing social variation in *L. malachurum* and within the subgenus as a whole. The principal components analysis excluding the solitary species and the socially aberrant *L. marginatum* populations show the *L. malachurum* populations separated based on an increasing proportion of workers with developed ovaries as populations are found more south, lending further support to the hypothesis of a north-south cline of decreasing eusociality.

ACKNOWLEDGMENTS

First and foremost, I would like to thank my supervisor Miriam Richards for the invaluable assistance with my research, the writing of my thesis, and also the opportunity to spend two months in Greece to conduct field research. This was so far, the best experience of my life. My time in Greece was both lonely and very challenging at times, but most of all, rewarding. I thank Janet Pinder for her assistance in the field in Greece and for a wonderful time in Rhodes! I thank Barba Mitso for feeding and watching out for me and for helping me dig my last two nests, without him I could not have done it! I would also like to thank Evangelia Skandalis and Zoe Pontikakou for a wonderful experience and their kind hospitality, and especially their Greek food!

I would like to thank the other members of my supervisory committee, Dr. Don Ursino, Dr. Mike Bidochka, Dr. Alan Castle, and especially Dr. Kevin Brown for their helpful comments. I thank Doug Yanega at the University of California for his personal correspondence regarding *Halictus rubicundus* and Adam Cronin at Hokkaido University for his personal correspondence regarding *Lasioglossum (E.) baleicum*. I thank Dr. Ian Brindle for the use of his analytical balance; Mike McConnell, Mike Spironello and Adonis Skandalis for the use of their computers; Dr. Bob Carlone, and lab group, for the use of his dissecting microscope. I thank Kevin Woodward and Jennifer Blahut for their help and support, as well as everyone else who has helped me along the way.

Finally, I give special thanks to Mitzi Seifried and Ross Beattie for reading and editing my thesis, and also for their moral support and friendship; my parents, family and friends (especially Amanda Bradford) for their support and encouragement during the last two years; and last but not least, Bob and Dave for their love and support.

TABLE OF CONTENTS

LIST OF TABLES

Chapter one

Chapter two

Appendix

LIST OF FIGURES

Chapter one

Chapter-two

INTRODUCTION

Altruism, in insect biology, is a behaviour that has been defined in terms of consequences rather than intentions, as would be the case in humans (Crozier and Pamilo 1996). Altruism can be defined as behaviour that enhances the reproductive success of relatives other than progeny (Michener 1974; Crozier and Pamilo 1996). For example, daughter workers that forgo reproduction and help in raising their mother's (the queen) offspring, i.e. their sisters. The emergence and maintenance of altruism are paradoxical evolutionary phenomena. Why did selection favour non-reproductive individuals, when it is through reproduction that genes for such behaviours are passed on (Michener 1974; Crozier and Pamilo 1996)?

Eusociality is observed when adults of more than one generation co-exist, among which there is cooperation in brood care and division of labour, with some individuals who do not reproduce (Michener 1974). The non-reproducing individuals within a eusocial society are members of the worker caste that help the queen in raising her offspring (the reproductives). In highly eusocial colonies, such as those observed in many ant species, colonies can grow to contain many workers that are extremely helpful and are completely sterile. Such workers exemplify the extreme of reproductive altruism. This classic example, however, is not fitting in less strongly eusocial societies, when varying degrees of altruism are observed, where only some members are altruistic while others reproduce. Likewise, an individual may help another, and later in life reproduce. These varying degrees of altruism have indeed been observed in many insect societies, accordingly, the study of insect eusociality is essential to understanding the origin and maintenance of altruistic behaviour (Michener 1974).

Eusocial behaviour has most often been documented in the Hymenoptera (ants, bees and wasps). In particular, within the family Halictidae (the sweat bees) and tribe Halictini, eusociality has evolved and reversed repeatedly (Packer 1991; Richards 1994; Danforth and

Eickwort 1997; Danforth 1999; Danforth et al., 1999) making these bees an ideal model for revealing the factors affecting the evolution of altruism and eusociality. Social organization of halictine colonies ranges from solitary nesting, to varying levels of societies, including semisocial, communal and eusocial societies, thus offering promising opportunities for comparative analyses to reveal the evolutionary, ecological and behavioural factors affecting the expression of altruistic behaviour.

To fully understand the factors that are most important for selection to favour altruism and eusociality, a detailed understanding of the social behaviour of species at different social levels is required. This is not easy to do because even within species, geographic and temporal variation in social behaviour exists. Therefore, it is important to understand the factors that lead to social variation between, as well as within, species. On a spectrum from weak to strong eusociality, *Lasioglossum (Evylaeus) malachurum* represents strong eusociality, and also exhibits geographic variation in colony social organization.

Current evidence suggests that *L. malachurum* is strongly eusocial (i.e. has more altruistic workers) in northern Europe and is more weakly eusocial in southern Europe (Knerer 1992; Richards 2000). The first objective of this thesis was to test the hypothesis of a north-south cline of decreasing eusociality by studying the colony social organization of a population that is further south than any previously studied population of *L. malachurum*. If the north-south cline of decreasing eusociality exists, then it is expected that *L. malachurum* in Greece should be weakly eusocial and workers should exhibit a low degree of altruism, as measured by a high degree of personal reproduction. If the hypothesis is unsubstantiated, then the Greek population is expected to exhibit strong eusociality and workers will exhibit a high degree of altruism, measured by a low degree of personal reproduction.

The second objective of this thesis was to determine which colony, demographic, and ecological factors were most closely associated with defining intraspecific social variation in *L. malachurum*. For instance, if it were found that a north-south cline of

decreasing eusociality does exist, then it would be the variable traits that must be important in defining the social level. Conversely, if the cline did not exist, then the invariable traits would be considered important to defining eusociality in *L. malachurum*.

Within the subgenus *Evylaeus* evolutionary reversals of eusocial behaviour to solitary nesting or social polymorphism have been reported, making this group particularly interesting for studies of social evolution (Packer 1991; Danforth 1999). The third objective of this thesis was to determine whether patterns of social variation within *L. malachurum* reflect patterns within *Evylaeus* as a whole. A comparative study was performed to determine which colony, demographic, and ecological characteristics tend to be associated with social variation in this subgenus. Are these the same traits that govern sociality within *L. malachurum*? If not, do the differences in characteristics distinguish *L. malachurum* from other *Evylaeus*? It is not until we fully understand the factors that govern intra- and interspecific social variation that discernment of the factors required for the evolutionary loss or gain of eusociality, and the maintenance of altruism can be revealed.

LITERATURE REVIEW

Bees and their social repertoire

From a human perspective, the most beneficial insects are the bees. For example, bees are important plant pollinators. Bees are members of the superfamily Apoidea, which consists of eight families and approximately 20,000 species (Michener 1974; Huffaker and Gutierrez 1999). Bees have differentiated from sphecid wasps by abandoning the wasp behaviour of feeding their larvae insect prey. Instead, bees feed their larvae with pollen or nectar collected from flowers or from glandular secretions derived from the same source. Bees are holometabolous insects, which means they undergo complete metamorphosis, from egg to larva to pupa to adult. The larvae are dependent on adults for provisioning (Michener 1974).

Social organization in bees ranges from solitary, communal and semisocial to various levels of eusociality (Michener 1974). Bees of several groups have independently evolved eusociality, thereby making this group of animals ideal for the study of the origins and maintenance of eusocial behaviour. A solitary nest is the simplest social organization and is characterized by an overwintered foundress that establishes a nest in the spring and produces a reproductive brood, without a worker caste. No contact is made between generations and this cycle is repeated by each generation of foundresses (Fig 1.1).

Eusociality is achieved when a division of labour is established between two overlapping generations (mother queens and daughter workers) that cooperate in brood rearing (Michener 1974) (Table 1.1). The terms primitively and highly eusocial refer to the extremes of eusocial colony organization. Highly eusocial bees, such as honeybees (*Apis mellifera*) are characterized by morphological, physiological and role-specific castes where many sterile workers are always present to help their mother raise her offspring. In these species, queens are unable to survive without workers as they have lost the structures for

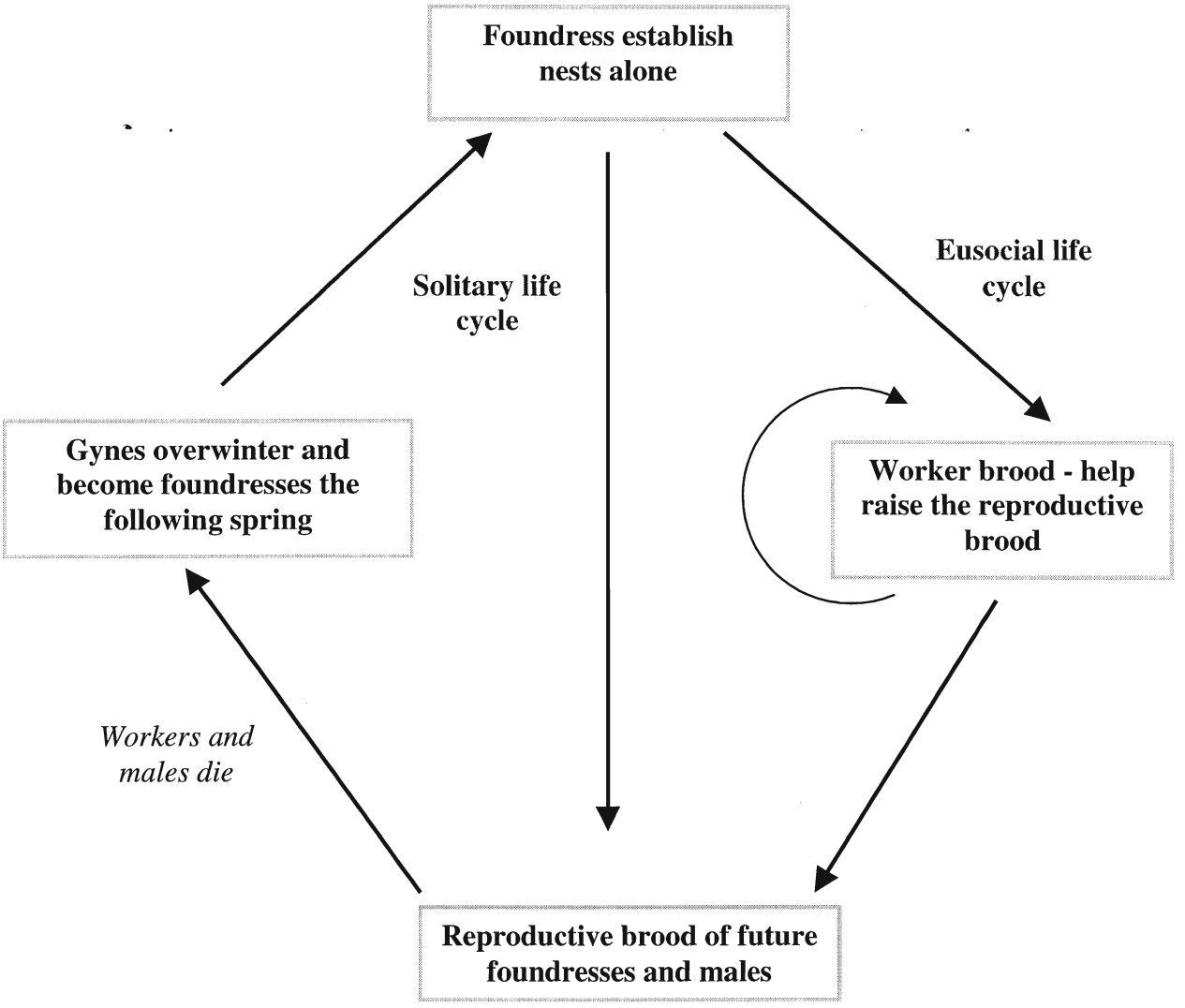

Figure 1.1: The annual life cycles of solitary and primitively eusocial sweat bees. Solitary life cycles are characterized by the absence of a worker brood. Eusocial life cycles can contain up to 5 worker broods before the production of the reproductive brood.

Table 1.1: Definitions for terms used to describe the various types of social organizations in bees.

Term	Definition	Examples
Solitary nesting	An overwintered foundress establishes a nest alone in the spring and produces brood alone. The generations do not overlap.	*L. (E.) villosulum* *L. (L.) mutilum* *L. (L.) scitulum*
Eusocial	A social system in which adults of more than one generation cooperate in brood rearing and which exhibit reproductive division of labour. Queens are the primary reproductives and workers help the queen in raising her offspring.	Mammals: Naked mole rat Insects: Many species of ants, termites, bees and wasps
Advanced or highly eusocial	A form of eusociality in which morphologically and physiologically differentiated castes exist. Workers are more or less sterile and always present to help their mother raise her offspring. Queens do not have the structures for pollen collecting; their sole function is egg laying. Queens are completely dependent on workers for the survival of the colony.	*Apis mellifera* (honey bee)
Primitively eusocial	Foundresses establish nests alone and produce a first brood of workers and sometimes males. Workers are morphologically and physiologically able to reproduce; they only differ from queens by their smaller body sizes.	*L. (E.) malachurum* *H. (H.) ligatus* *L. (E.) duplex* *L. (D.) zephyrum*
Obligately eusocial	Describes bees that always exhibit eusocial colony social organization.	*Apis mellifera* *L. (E.) malachurum* *H. (H.) ligatus*
Facultatively eusocial/ socially polymorphic	Describes bees that exhibit solitary nesting or eusocial nesting either in two different populations or sometimes within the same population. A foundress establishes a nest alone and may produce a first brood of workers or a first brood of reproductives	*L. (E.) albipes* *L. (E.) calceatum* *L. (E.) apristum* *Augochlorella striata*
Strong eusociality	A term used to describe the strength of colony eusociality. Strong eusociality is observed when most workers act altruistically, where few are reproductively active and many are highly helpful in nesting activities such as foraging, nest construction and nest defense.	*L. (E.) malachurum* *L. (E.) marginatum* *L. (E.) lineare*
Weak eusociality	A term used to describe the strength of colony eusociality. Weak eusociality is observed when few workers act altruistically and many lay their own eggs.	*H. (H.) sexcinctus* *H. (H.) farinosus*

pollen collecting; their sole function is egg laying. Due to the loss of the proper structures, workers are physiologically unable to mate and produce diploid eggs. They are only capable of laying trophic or male eggs (Michener 1974). Highly eusocial workers therefore exhibit behaviours that exemplify extreme reproductive altruism.

Primitively eusocial bees are characterized by a solitary phase at the beginning of the life cycle where an overwintered female establishes and provisions a first brood of workers alone (Fig. 1.1; Table 1.1). The workers are morphologically indistinguishable from queens except for size dimorphism; queens are larger. The workers usually remain in their natal colony and help their mother (queen) to raise the next brood(s), which consists of either workers or future queens and males (Michener 1974). Primitively eusocial workers are physiologically and morphologically able to mate and lay haploid or diploid eggs.

Workers that are mated with developed ovaries can lay diploid eggs and those with developed ovaries but which are unmated can lay haploid eggs (Michener 1974; Crozier and Pamilo 1996; Trivers and Hare 1976). The number of eggs laid by a worker is inversely proportional to the degree of altruism; the degree of altruism is therefore variable. For this reason, it can be used to assess the strength of eusociality of a species or population, strong eusociality being characterized by high levels of altruism (i.e. few workers lay eggs) and weak eusociality by low levels of altruism (i.e. numerous workers lay eggs).

The family Halictidae, the sweat bees, which earned their name from lapping the sweat off the skin of mammals (Michener 1974), is of particular interest because of their range and variability of social behaviour, among genera, species and populations. Their social repertoire can range from solitary to varying degrees of eusociality, and flexibility in social variability appears to have evolved independently among species.

There are two types of primitively eusocial sweat bees, facultatively and obligately eusocial. Facultatively eusocial sweat bees exhibit socially polymorphic behaviour, where some populations are solitary while others are eusocial, as in *Augochlorella striata*.

Obligately eusocial halictids (e.g. *H. ligatus* and *L. malachurum*) always produce a worker brood and exhibit two distinct castes, reproductive queens and worker daughters, but the degree to which workers exhibit altruistic behaviour can be variable across a species' range.

In facultatively eusocial species the production of the worker caste may be under environmental influence in some species, but genetic influence in others. The question raised by Eickwort et al. (1996) and applicable to all facultatively eusocial species is whether "solitary behaviour is based on an evolutionary loss of worker behaviour or on facultative expression of different social phenotypes under different environmental condition". For example, in Japan, variation in day length and temperature appear to be the most significant environmental factors influencing the social status of *L. calceatum* (Scopoli) and *L. apristum* (Vachal). These species exhibit solitary nesting at high altitudes where no worker brood is produced, and a eusocial one in the lowlands where the longer and warmer summers facilitate the production of a worker brood prior to the reproductive brood (Sakagami and Munakata 1972; Miyanaga et al., 1999). Similarly, *Halictus rubicundus* (Christ) is solitary in Colorado at high altitudes (Eickwort et al., 1996) but eusocial in New York (Yanega 1989, 1990, 1993). Even a single population might contain both solitary and eusocial nests, as was the case for *Augochlorella striata* in Cape Breton, Nova Scotia, a location that represents the northern limit of its range (Packer 1990). For *A. striata* in Nova Scotia, worker production and behaviour appears to be facultative and dependent on ecological and demographic factors (Packer 1990). The existence of both solitary and eusocial nests during the summer of 1987 was suggested to be the result of unusual drought conditions that resulted in high brood mortality and cessation of cell construction and foraging activity due to extremely hard soil conditions. Environmental factors alone, however, cannot fully explain facultatively eusocial behaviour in some species. Common garden experiments performed in the laboratory using the facultatively eusocial species *L.*

albipes, showed that solitary foundresses from the east of France did not completely convert to eusocial behaviour, and eusocial foundresses from the west of France did not convert to solitary behaviour, when placed in each other's temperature and light regimes (Plateaux-Quenu et al., 2000). This suggests that genetic factors also play a part in the behaviour of this socially polymorphic species.

Unlike facultatively eusocial species, obligately eusocial sweat bees do not ever switch to a solitary lifestyle, but can exhibit behavioural plasticity from one population to another or even from one year to the next within the same population. A detailed description of social variability in *L. malachurum* is presented in Chapter 1 and therefore will not be considered further here. *Halictus ligatus* has been well studied in southern Ontario (Packer 1986c,d; Richards et al., 1995; Richards and Packer 1995, 1996, 1998) and South Carolina (Dunn et al., 1998). A north-south cline of decreasing sociality exists, where populations in Ontario exhibit stronger eusocial behaviour (i.e. fewer workers are mated and have developed ovaries) than populations in South Carolina, which exhibit more weakly eusocial behaviour (i.e. more workers are mated and have developed ovaries). Furthermore, in the southern Ontario population, annual variation in local climatic conditions appears to affect the proportion of reproductive workers and thus the strength of colony eusociality. For example, in 1990, harsh environmental conditions resulted in the production of relatively small workers and a greater size difference between queens and workers (Richards and Packer 1995, 1996). This in turn led to a reduced proportion of reproductive workers since queens could effectively dominate workers and control their behaviour, resulting in a more strongly eusocial population. In contrast, under warmer and drier conditions during 1991, the size differential between queens and workers was reduced, a greater number of workers were reproductive, and as a result, the population was more weakly eusocial (Richards & Packer 1995, 1996).

Hypotheses to explain the maintenance of worker altruism

Altruism has been defined as behaviour in which an individual incurs some fitness cost by helping another, while the recipient of the act incurs some fitness advantage (Lin and Michener 1972). In sweat bees, the degree of altruistic behaviour is measured by how much a worker helps her nest mates at the cost of her own direct reproduction. The key evolutionary question is: why should a worker capable of reproducing stay and help her mother instead of leaving her natal nest to establish one of her own? Three main hypotheses have been proposed to explain the origin of eusociality and the maintenance of altruism: mutualism (Lin and Michener 1972), kin selection (Hamilton 1964, 1972), and parental manipulation (Michener 1974; Alexander 1974).

Mutualism occurs when both the helper and the recipient receive a direct fitness benefit from their interactions (Lin and Michener 1972), implying that both parties must cooperate equally to ensure a fair trade-off. For this hypothesis to apply to primitively eusocial sweat bees, workers must be laying their own eggs. In this case, the queen benefits by having helpers provision and raise the reproductive brood, thereby increasing her reproductive fitness, while the helpers benefit by reproducing and increasing their fitness directly. For mutualism to evolve, close relationships among nest mates are not required (Lin and Michener 1972).

Hamilton (1964) proposed 'kin selection' as a mechanism for the evolution of social and altruistic behaviour. Kin selection is defined as the evolutionary effects of both parental aid given to descendant kin (offspring) and altruism directed to non-descendant kin (relatives other than offspring). Hamilton explained that the evolution of altruism rests on the premise that the unconscious goal of reproducing, from an evolutionary perspective, is to propagate one's distinctive alleles. Personal reproduction achieves this ultimate goal in a direct fashion, but helping genetically similar relatives survive to reproduce, provides an indirect route to the same goal. The key factor promoting kin selection is whether an

individual can pass on more copies of its genes by helping relatives reproduce (indirect selection) than it could by reproducing individually instead (direct selection).

In the context of kin selection, the motivation (i.e. fitness benefit) for an altruist is the close relatedness to his or her relatives. The coefficient of relatedness (r) is the probability that two individuals both possess the same rare allele by virtue of inheriting it from a recent common ancestor. For helping behaviour to be adaptive, the following condition must be met: $rb-c > 0$, where b is the benefit to the recipient in terms of the increase in the number of kin raised due to help, c is the cost to the altruist in terms of the number of offspring lost and r is the coefficient of relatedness between altruist and the kin she raised (Hamilton 1964, 1972; Trivers and Hare 1976). Therefore, it would be advantageous for a worker to forgo direct reproduction and help her mother if such behaviour resulted in more replicates of her genes in future generations than she could have produced through her own offspring (Lin and Michener 1972).

Hymenopterans have a haplodiploid sex determination system whereby females are produced from fertilized eggs and males are produced from unfertilized eggs. When the queen has mated only once, she produces daughters who share by descent three-quarters of their genes (sister-sister coefficient of relatedness is 0.75). This high degree of relatedness would encourage sisters to raise sisters rather than their own daughters to whom they are less related ($r = 0.50$). By helping raise their sisters, they actually increase the number of similar genes passed on to the next generation, thus increasing their inclusive fitness. However, since workers are related to their brothers by only $r = 0.25$, and to their sons by $r = 0.50$, for worker altruism to be favoured, both a high degree of relatedness among offspring (same father) and a biased sex ratio in favour of females is advantageous. For example, in central New York, *Augochlorella striata* queens mate only once and sister-sister relatedness is three times higher than sister-brother relatedness. The relatedness asymmetry

coupled with a 3:1 sex ratio bias in favour of females, creates genetic incentives favouring worker behaviour in this population (Mueller 1996).

If kin selection is important for the maintenance of worker altruism, then factors that influence the degree of relatedness within a colony would have great significance for worker behaviour. For example, multiple mating by queens, which has been documented in many eusocial Hymenoptera including sweat bees (Arévalo et al., 1998; Crozier & Pamilo 1996; Keller 1997; Knerer 1992; Ross 2001), presents a problem when explaining the maintenance of altruism because sister-sister relatedness may be less than or equal to mother-daughter relatedness due to multiple paternity. If workers are more related to their own offspring than they are to their sisters, then direct reproduction would result in higher inclusive fitness and workers should be less altruistic. Polygyny (multiple-foundress associations) also affects intra-nest relatedness and potential resource allocation, because workers are potentially unrelated to the offspring produced by different mothers. Workers laying male eggs also affects intra-nest relatedness and workers would be more likely to devote more energy into raising their own sons rather than the queen's sons (their brothers) (Crozier and Pamilo 1996).

Queen manipulation of workers by physical force has provided a proximate explanation to account for helping and non-reproducing worker behaviours (Crozier and Pamilo 1996), and has been documented in sweat bees (Michener 1974; Kukuk and May 1991; Richards and Packer 1996; Richards et al., 1995). Size dimorphism between queens and workers is one important factor governing how well a queen is able to control worker behaviour. In the facultatively eusocial species, *L. calceatum* and *L. albipes*, the proportion of males produced is negatively correlated with queen- worker size dimorphism, that is, the smaller the size difference between the castes, the greater the number of males produced by workers early in the colony cycle (Knerer 1980; Plateaux-Quenu 1992; Plateaux-Quenu et al., 2000). In the obligately eusocial *H. ligatus*, the larger queens physically dominate

smaller subordinates in multi-foundress nests (Richards and Packer 1996). Furthermore, when the size difference between a queen and her workers is small, an increased proportion of workers lay eggs in the reproductive brood (Richards and Packer 1995, 1996). Similarly, in laboratory reared *L. zephyrum*, queen aggression ensures the inhibition of worker ovarian development. In this species caste differentiation is determined through behavioural differences and larger females become queens through aggressive superiority (Kukuk and May 1991).

How long the queen lives may also be an important factor affecting worker behaviour (Michener 1974; Breed 1976). Queens that die prior to the reproductive brood provide opportunities for workers to gain reproductive advantage. In some cases, a mated worker may take over the duty as the colony's primary egg layer, cease foraging and thus becoming the 'replacement queen', while the other workers in the nest continue to provision and forage. In other cases, for example late in the colony cycle, all workers within the nest may be actively involved in egg laying, and the colony social organization, in effect, switches from eusocial to communal (Michener 1974).

The strategy adopted by an individual worker may not just be a consequence of the degree of relatedness to other members within their colony, or queen manipulation, but also a function of timing. By the time workers emerge from their brood cells, it may be too costly or risky for workers to build and provision their own nests, due to limited nesting sites and hardened ground conditions (Yanega 1992; Wcislo, in Choe and Crespi 1997). Under such conditions, workers that remain in their natal nest and help their mother raise her offspring may be utilizing a better strategy than if they were to leave and risk unsuccessful nest establishment or death. Furthermore, a worker may gain opportunities for direct reproduction in the future if the queen dies. In *L. malachurum*, repression of worker reproduction is only partially effective in the last brood. At that time, some workers from the first and second broods produce some or all of the males while the queen retains

reproductive monopolization of gyne production (Knerer 1980; Packer and Knerer 1985; Knerer 1992).

Phylogenetic approaches to the evolution of eusociality in sweat bees

Phylogenetic approaches are the most effective method of examining the direction of social evolution in sweat bees (Packer 1991; Packer 1997; Richards 1994; Danforth and Eickwort 1997; Danforth 1999; Danforth et al., 1999). In the subfamily Halictinae, there are eight genera and subgenera that include solitary, eusocial and socially polymorphic species. These are *Halictus (Halictus), Halictus (Seladonia), Lasioglossum (Evylaeus), Lasioglossum (Dialictus), Lasioglossum (Lasioglossum), Augochlorella, Augochlora,* and *Pereirapis* (Michener 1974). Genetic data have contributed to the development of more accurate phylogenies, and using behavioural data collected from members of each genus, characteristics have been mapped onto phylogenies to reconstruct the origin and path of eusociality in these groups of sweat bees. Such phylogenetic analyses help to identify those forces, whether intrinsic or extrinsic, that led to the adoption of one type of life cycle (e.g. eusocial) over another (e.g. solitary nesting).

Phylogenetic studies of sweat bees have revealed that eusociality appears to be the ancestral state in species belonging to two subgenera of the genus *Lasioglossum* (*Evylaeus* and *Dialictus*). Reversals to solitary behaviour have arisen independently five times while socially polymorphic behaviour has evolved independently three times (Packer 1991; Packer 1997; Danforth 1999). In the third subgenus, *Lasioglossum (Lasioglossum)*, solitary behaviour has been the only behaviour observed (Packer 1991; Danforth 1999). In *Halictus*, loss of eusocial behaviour to solitary behaviour has occurred once and losses to facultatively eusocial behaviour has occurred three times (Richards 1994; Danforth et al., 1999).

Social polymorphism may have resulted from the evolution of a flexible worker caste that varies by the degree of reproduction depending on local environmental and/or

demographic circumstances (Danforth et al., 1999). Alternatively, reversals to solitary behaviour have been attributed to the omission of the worker caste due to geographic limitations (Packer 1997; Miyanaga et al., 1999). Many sweat bees species have not been identified or remain unstudied (Wcislo and Danforth 1997). Of those that have been studied, many exhibit some form of geographic variation including obligately eusocial species, such as *H. ligatus* and *L. malachurum*, leaving the question of whether the variation is genetically fixed or environmentally mediated (Cronin, unpub. manuscript). The interpretation of the evolution of social behaviour, therefore, remains dynamic and as new information regarding social behaviour is revealed, this interpretation may become clearer.

CHAPTER ONE

Social biology of *Lasioglossum (Evylaeus) malachurum* Kirby (Hymenoptera: Halictidae) in southern Greece

Sweat bees are the most behaviourally variable of the bees because they exhibit a range of social behaviours, from solitary to strongly eusocial. There are probably thousands of sweat bee species, of which only a small fraction has been studied in any detail. *Lasioglossum malachurum* has stood out because it is one of the most abundant and widely distributed sweat bees in Europe and has one of the most strongly differentiated caste systems. Queens are much larger than workers and monopolize reproduction of their colony, making this species one of the most strongly eusocial sweat bees studied to date (Michener 1974; Knerer 1992). In addition, it exhibits geographic variation in nesting and social behaviour that appears to be correlated with a north-south cline of decreasing eusociality (Knerer 1992; Richards 2000). The social characterization of *L. malachurum* is based on field studies carried out on different populations in Europe. Knerer (1973, 1992) studied populations in the Isle of Wight, England; Tulln, Austria; Dordogne, Marseilles, Longjumeau, France; Estepona, Spain, Eforie Nord Romania and Platamon Greece. Knerer suggested that *L. malachurum* is a paradigm of a strongly eusocial sweat bee because it produces up to three worker broods in the absence of males and very few workers reproduce (i.e. workers are not mated and do not have well-developed ovaries). Knerer's (1973, 1992) descriptions of the nesting and social biology of *L. malachurum*, however, are incomplete and not always clearly reported.

Richards' (2000) characterization of *L. malachurum* in southern Greece was based on a compilation of five years of study: 1989, 1994, 1996-1998. Her data were pieced together based upon the assumption that annual variation in the timing of events from year to year did not occur due to predictable hot and dry summers in Greece. Data regarding

worker reproduction were based on workers and foundresses collected from flowers and also from four nests excavations conducted in June 1998. Richards' (2000) study revealed a different picture than that of Knerer's (1992) description of *L. malachurum* in southern France. No queens and only one male were found from the four nests excavated at the end of June and 75% of workers were mated and up to 70% of workers had well-developed ovaries, data that suggested that *L. malachurum* was weakly eusocial in Greece.

It was my objective to (1) obtain a more complete picture of the nesting and social biology of a southern Greek population in order to investigate intraspecific social variation and test the hypothesis of a north-south cline of decreasing eusociality, and (2) to discern which colony, ecological and demographical factors are most closely associated with intraspecific variability. Having an accurate portrait of the social biology of *L. malachurum,* and its strength of eusociality, will contribute to our understanding of the possible factors associated with this species' geographic variability as well as the evolution of social behaviours in sweat bees. I begin with a review of the life history of *L. malachurum.*

L. malachurum life history

The life cycle of *L. malachurum* is most distinctly different from most other halictines because of an almost perfect gradient of increasing numbers of worker broods in populations from north to south (the exception is southern Spain in which only two worker broods are produced). The number of worker broods is an important characteristic that may influence colony demography and eusociality because more helpers increase production of the reproductive brood, and therefore colony size, and thus the reproductive success of the foundress female.

Caste differentiation in *L. malachurum*, based on size and behaviour of queens and workers, is one of the criteria that has led to this species' designation as strongly eusocial (Knerer 1992). Queens are significantly larger than workers, with almost no size overlap

between castes. Once the first brood of workers emerges, the queen remains in the nest and monopolizes egg laying (Michener 1974; Knerer 1992). According to Knerer (1992), *L. malachurum* egg layers choose the caste of female offspring (workers or gynes) at the time of brood cell construction and provisioning. Gyne-destined cells are larger than those of males and workers, and the pollen mass is saddle-shaped, while the pollen masses of both workers and males are small, spherical shaped. Furthermore, at the time of eclosion, gynes, but not workers, contain massive fat bodies in their abdomens to ensure their survival for the ensuing diapause, which can last up to 7 months (Knerer 1992). It is possible that the composition and quality of provisions allocated to workers and gynes differ and thus determine caste, which is possibly the case for *H. ligatus* in Ontario. Gyne pollen masses were significantly heavier and contained more sugar than those of workers or males and increased sugar content was suggested to influence the amount of fat deposition in developing brood and allow gynes to survive their diapause (Richards and Packer 1994).

The general life cycle of this species is essentially the same in all localities. Overwintered foundresses emerge from their hibernacula in early spring and begin searching for suitable nesting sites. The initial stage of the colony cycle represents the solitary phase (Michener 1974; Knerer 1992; Smith and Weller 1989). The occurrence of polygynous nest founding (multiple foundresses in the same nest) is extremely rare in this species. The nesting sites are mostly located in sparsely vegetated areas where aggregations can contain hundreds of nests built in close proximity (Knerer 1973, 1992; Smith and Weller 1989; Richards 2000). All nests are subterranean and can be identified by a concentric tumulus around the nest entrance.

Knerer (1973) reported that nest initiation by foundresses takes place in two phases. Smith and Weller (1989) showed that this bi-phasic period was a result of high conspecific nest usurpation by nestless foundresses. The first phase represents those females that successfully found a suitable nesting site and began to forage. The second phase is

identified by the activity of potential nest usurpers. These foundresses fly around the aggregation and enter nests left temporarily vacant by foraging owners (Smith and Weller 1989; Kaitala et al., 1990). The usurper remains at the entrance until the nest owner returns, at which time either the usurper leaves immediately or a fight ensues. Most often (80%) the nest owner wins the fight (Smith and Weller 1989).

After nest initiation, foundresses forage and construct cells for the first brood of workers. The development of brood 1 coincides with a quiescent, or inactive, period when no activity is seen outside the nest. Upon the emergence of the brood 1 workers, the queen stays in the nest and remains the principal egg layer, while the workers forage and provision the second brood. A second quiescent period coincides with the development of brood 2. In England, the second brood consists of gynes and males that mate. The gynes overwinter to repeat the cycle the following year while males, workers and queens die. In central locales, the second brood consists of workers and the third brood of the reproductives. In southern locales the second and the third broods consist of workers while the third and fourth contain reproductives (Knerer 1992; Richards 2000).

When *L. malachurum* reproductives emerge in late summer, mating between gynes and males can occur on or near flowers (Knerer 1992). Ayasse et al. (1990) suggested that monandrous mating is the rule, but Knerer (1992) suggested that multiple matings are common based on laboratory experiments of field caught males and gynes. Sex pheromones play an important role in the mating biology in *L. malachurum* to the extent that males can distinguish between, and are more attracted to, young unmated gynes than to young but mated gynes, old queens and workers (Ayasse et al., 1990; Ayasse et al., 1993; Ayasse et al., 1999).

Materials and methods

The study site was located in the village of Agios Nikolaos Monemvasias (ANM), which is located near the southeastern tip of mainland Greece (Fig 1.2). The village is surrounded by numerous olive groves situated on terraces, carved out from the mountainsides (Fig. 1.3a). This area experiences typical Mediterranean weather where winters are cool and rainy and summers are long, hot and very dry. The ground is very rocky and soils are predominately red clay, which becomes extremely hard during the dry summer months (Richards 2000).

Field studies were carried out for seven weeks, from 10 May to 25 June 2000, during which time two nesting aggregations were found (MALA1 and ANM). Nests were identified by tumuli that formed a circular ridge around the nest entrance. The nest entrance was the same diameter as a bee's head width (approx. 2.2 mm), situated in the center of the tumulus. Some nests had a 'tower-like' structure as the nest entrance that may have been a result of the wind blowing away the dirt of the tumuli, leaving the top part of the nest tunnel exposed. Dr. Miriam Richards located the first aggregation, designated MALA1, in 1998 when it consisted of 42 nests in a dirt road on a hillside above ANM. In mid-summer 1999, MALA1 was not found, likely due to road grading (Richards 2000). In 2000, this aggregation of nests was again located along the roadside and, in addition, numerous nests were also found on the terraces below the road (Fig. 1.3b).

In 2000, a total of 133 *L. malachurum* nests (Fig. 1.3c) were marked at MALA1 with coloured flags made of tape and souvlaki sticks on which nest numbers were written in indelible ink. The terraces were flat ledges formed out of the mountain, approximately 112

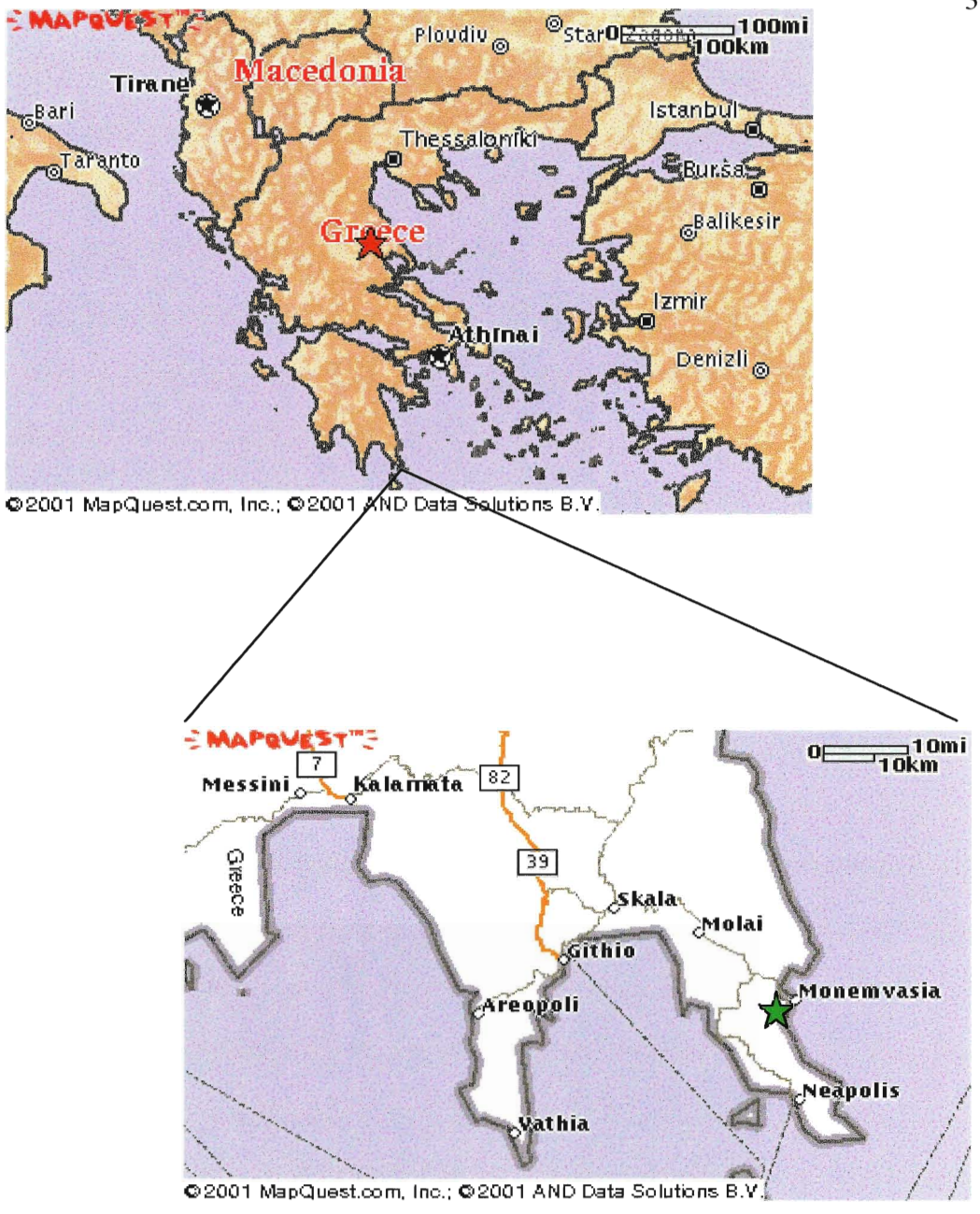

Figure 1.2: Map of Greece showing the location of the study site (green star), Agios Nikolaos Monemvasias, located near the southeastern tip of mainland Greece.

x 4 m in size. Nests were scattered along four terraces and the road above, so not all the nests in this aggregation were marked. Although most nests were quite far apart from each other (51% of nests were ≥20 cm apart), some were in clusters of two or three, separated by only a few centimeters. A tape measure was used to measure nearest-neighbour distance, defined here as the straight-line distance from the entrance of one nest to the entrance of its nearest neighbour.

The second aggregation, designated ANM, was also located in the village, approximately 200 meters north of the house at which I stayed. At ANM 203 nests were located and marked in an unused garden (25 x 6 m), which contained two large olive trees situated along one edge of the site. The ground was covered with both small and large rocks and the soil was red and clay-like. Unlike the MALA1 aggregation, nests formed a very dense aggregation in the upper half of this site. In the lower half of the garden, nests were less dense, usually in clusters of three or four. Nearest-neighbour distances were measured for 157 nests located in the upper half of the site.

Observations and collection of foragers

Initial behavioural observations were performed at MALA1. The minimum number of foragers per nest and the proportion of nests with guards were determined from nests that were observed for a minimum of thirty minutes, since good estimates of the number of foragers per nest could be done in that time. Thirteen nest surveys (2 partial) were performed at the MALA1 aggregation in order to determine the number of active nests each week; (two full and one partial surveys during week 1, two full and one partial during week 2, one during week 3, two during week 4, one during weeks 5 and 6 and two during week 7). Nest surveys entailed a walk about the aggregation and marked nests were recorded as to (1) whether they were open or closed (indicated by a dirt plug in the entrance), (2) had a guard at the nest entrance, (3) evidence of foraging activity and (4) the presence, and type, of

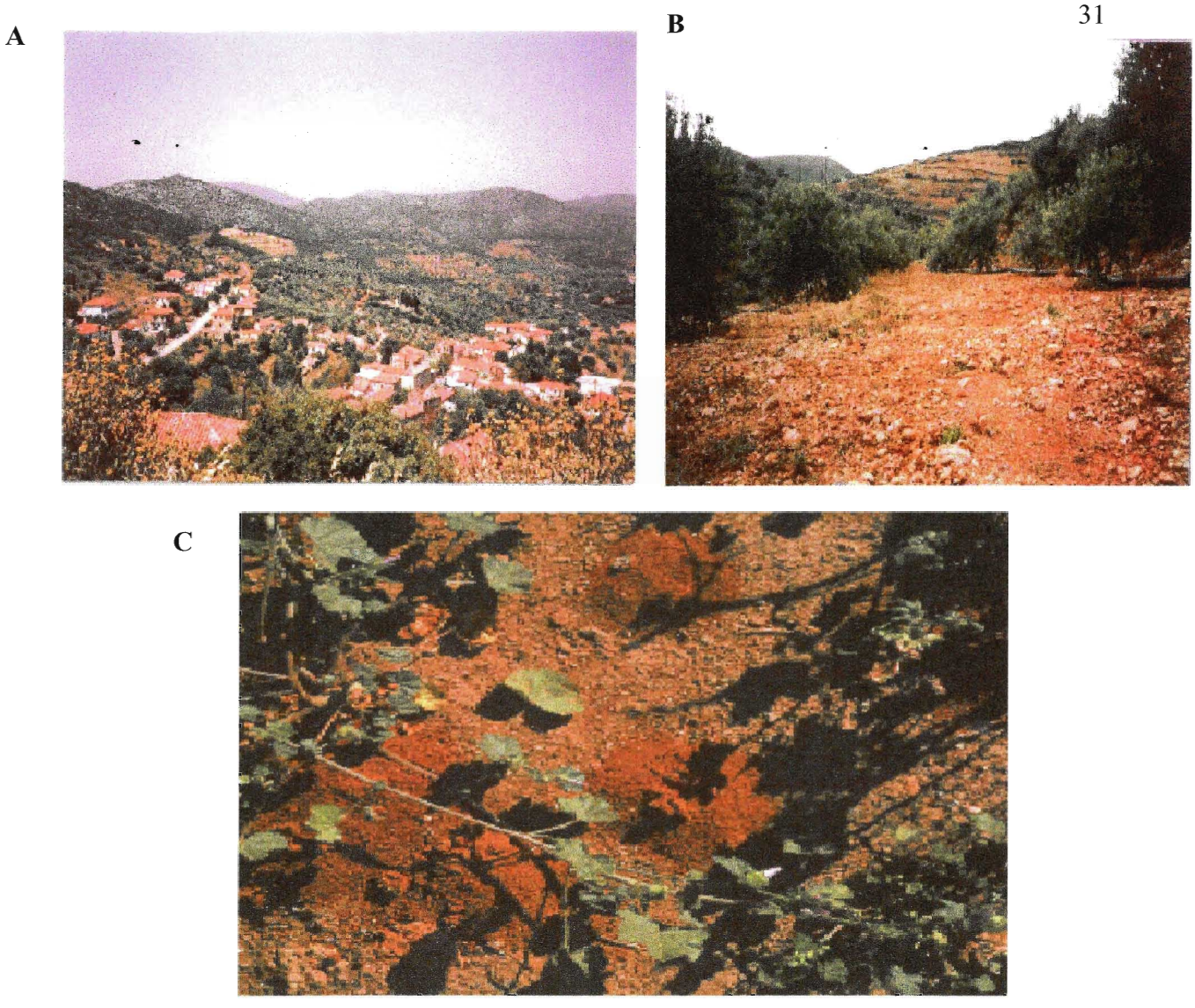

Figure 1.3: The study site. (A) The village of Agios Nikolaos Monemvasias. The village was surrounded by numerous olive groves that were situated on terraces, which were carved out from the mountainside. (B) The MALA1 aggregation located on 4 olive grove terraces and the roadside above. (C) A topographic view of a *L. malachurum* nest. The excavated dirt forms a tumulus around the nest entrance.

parasites close to or entering nests. Fresh dirt piled around the nest entrance (tumuli) suggested that the nest was active. Dried up tumuli indicated no recent digging and suggested that no activity was occurring in or outside of the nest.

To determine the minimum number of foragers per nest at MALA1 and ANM, foragers were collected while leaving their nests. Bees were caught by placing a clear plastic cup over the nest entrance. The bees were then immediately transferred using a hand net from the cup to containers of 100% ethanol. The cup was removed to allow arriving bees to enter the nest.

Nest excavations

In total, twenty nests were excavated at MALA1 (n= 3) and ANM (n=17). Nest excavations were initiated by blowing white talcum powder down nest entrances to visualize and follow the tunnels downwards. A shovel was used to remove the dirt in front of the nests, a chisel was used to remove rocks and a hand shovel and a knife were used to remove small amounts of dirt around the tunnels. All fully excavated nests (n=12) were dug to approximately 5 cm below the end of the nest to ensure that all nest contents were found. All adults (dead or alive), parasites, eggs and provision masses, and developing brood were removed and immediately placed in 100% ethanol. To determine sex and to record size measurements, unharmed developing brood were removed from their brood cells and placed in wax-lined petri dishes, where they were raised to adulthood prior to their storage in 100% ethanol. In 8 cases, nests could only be partially excavated because parts of tunnels were filled with dirt and so could not be followed.

Identification of age, caste and sex

Figure 1.4a shows a newly emerged male, an adult worker and a newly emerged gyne. Males can be identified by their small size, narrow heads and bodies, an extra

Figure 1.4: Developmental stages of adults and pupae. (A) From left to right, a newly emerged male, an adult worker and a newly emerged gyne at 10X magnification. (B) Seven of nine pupal stages of *L. malachurum* (7X magnification): (1) white pupa, the first stage after pupation; (2) pink-eyed pupa; (3) red-eyed pupa; (4) black-eyed pupa; (5) ¼ pigmented pupa; (6) ¾ pigmented pupa and (7) fully pigmented pupa, the last pupal stage. Pupal stages 1-4 refer to the pigmentation of the eyes, while stages 5-7 refer to pigmentation of the body.

antennal annulus (males have 13, whereas females have 12 segments) and, in most cases, by the yellowish colouration on their legs, antennae and abdomens. Gynes (future queens) can be distinguished from workers by their significantly larger body size, little to no wing or mandibular wear, and by the timing of emergence (gynes were present in brood(s) produced in late summer). Queens could be distinguished from workers and gynes by their severe wing and mandibular wear, having sperm in their spermatheca and having well developed ovaries or ovaries that had degraded after egg laying. Queens could be further distinguished from workers based on their larger body sizes (Richards 2000; Knerer 1992). Workers were considered adults if they were caught outside of their brood cells (e.g. foraging or in the nest tunnel). Imagos were fully developed (eclosed) bees that were still in their brood cells.

It was possible to determine sex and caste type if the brood survived to the pupal stage (Fig. 1.4b). Sex was determined either at the time of excavation, if pupae were pickled immediately, or after development to a pupal stage in a petri dish. Small, medium and large larvae could not survive outside of their brood cells and so they were preserved immediately in ethanol. Fifty-six fully-grown larvae and prepupae were raised in wax-lined petri dishes to a pupal stage or to an imago.

Dissections of adult females

Adult females were dissected to determine reproductive status, which was based on matedness and the stages of ovarian development (Fig. 1.5). Dissections were performed using a Leica Wild M3Z® dissecting microscope. Matedness was determined by the presence or absence of sperm in the spermatheca. Ovarian development was scored by assessing the size of each developing oocyte in an ovary relative to a fully developed oocyte (i.e., 1/4, 1/2, 3/4, 1), and then summing these fractions. A score of zero was assigned to ovaries that were clear, flat and thin. A total score of 0.1 was assigned to bees that had nurse cells or oocytes that were too small to score, and bees whose ovaries were only

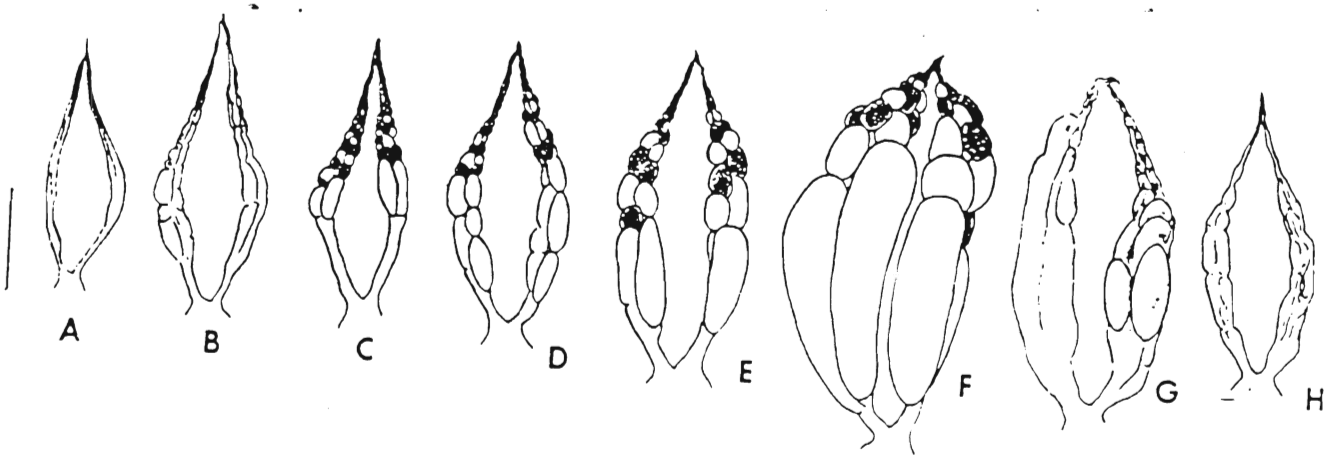

Figure 1.5: Stages of ovarian development. Ovarian development was scored based on the size of the developing oocytes from: (A) undeveloped ovaries with a total ovarian score of 0; (B) ovaries that were slightly thickened or with nurse cells too small to score, a total ovarian score of 0.1; C-F represent ovaries that contain oocytes that are ¼ (score=0.25), ½ (score=0.50), ¾ (score=0.75) and fully developed (score=1.0), respectively. Total ovarian development scores represent the summation of individual oocytes scores. (G) and (H) show the degradation of the ovaries after egg laying (total score=0.1) (Diagram from Michener 1974, pg. 290).

slightly thickened. Degraded ovaries, which are a result of post-egg-laying, were assigned a score of 0.1.

Wear and tear scoring

The degree of mandibular and wing wear of adult females was used to assess the ages of adult females and to help in differentiating among queens, workers and gynes. Mandibular wear (Fig. 1.6a,b) was scored from 0-5. A score of 0 represented sharp, defined mandibles and 5 represented very blunt mandibles with the sub-apical mandibular tooth indistinguishable. Wing wear was scored on a scale from 0, forewings with the distal edge completely undamaged, to 5, forewings with distal edge mostly or completely torn away by nicks and tears (Fig. 1.6c,d). Total wear score represents the sum of wing and mandibular wear for each individual.

Body size measurements

Head width measurements, standard and reliable measures of size for both pupae and adult bees, were used to assign caste and to calculate size differentials between queens and their offspring (Richards and Packer 1996). All adult workers, queens, male and female brood that had reached pupal stage and were not damaged, were measured at 32X magnification using a Zeiss Stemi SVII® binocular dissecting microscope fitted with an eyepiece reticle. Head width was defined as the distance across the widest part of the head including the compound eyes (Fig. 1.7a). Wings were removed from specimens, taped to paper, and measured from the costal vein on the left wing (Fig. 1.7b). If the left wing was damaged, I measured the right wing.

Caste size dimorphism was expressed as:

$$\frac{\text{Queen head width} - \text{Worker head width}}{\text{Queen head width}} \times 100$$

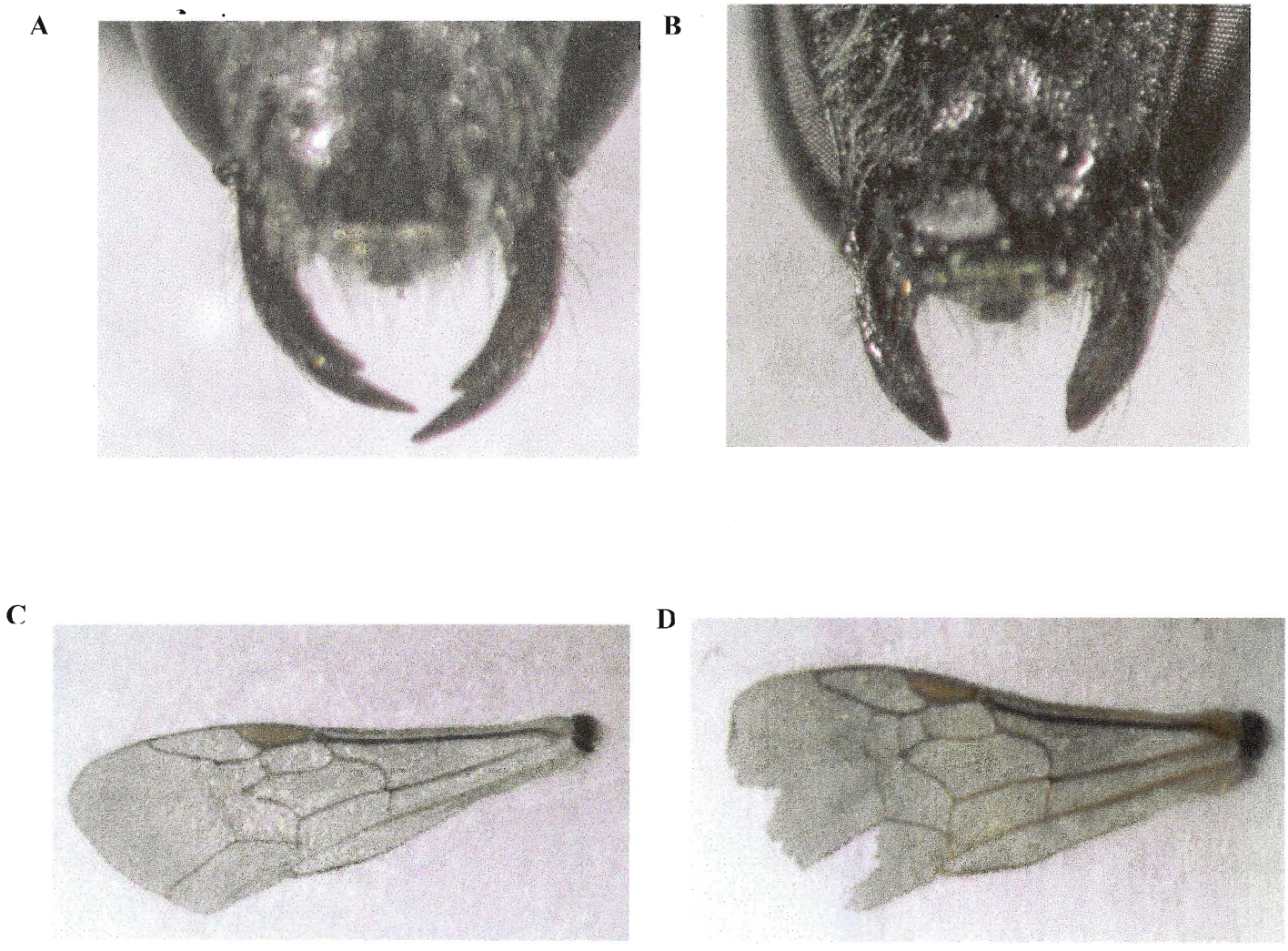

Figure 1.6: Mandibular and wing wear in adult *L. malachurum* females. Mandibular and wing wear were scored on a scale from 0 to 5. (A) A worker, with a mandibular score of 0, had mandibles that were sharp with two well-defined supra- and sub-apical teeth. (B) A queen, with a mandibular score of 5, had mandibles that were very dull and the sub-apical tooth had completely worn away. (C) A worker's wings that had no nicks or tears and were completely undamaged, representing a wing wear score of 0; (D) A queen's wing that was severely torn, representing a wing wear score of 5.

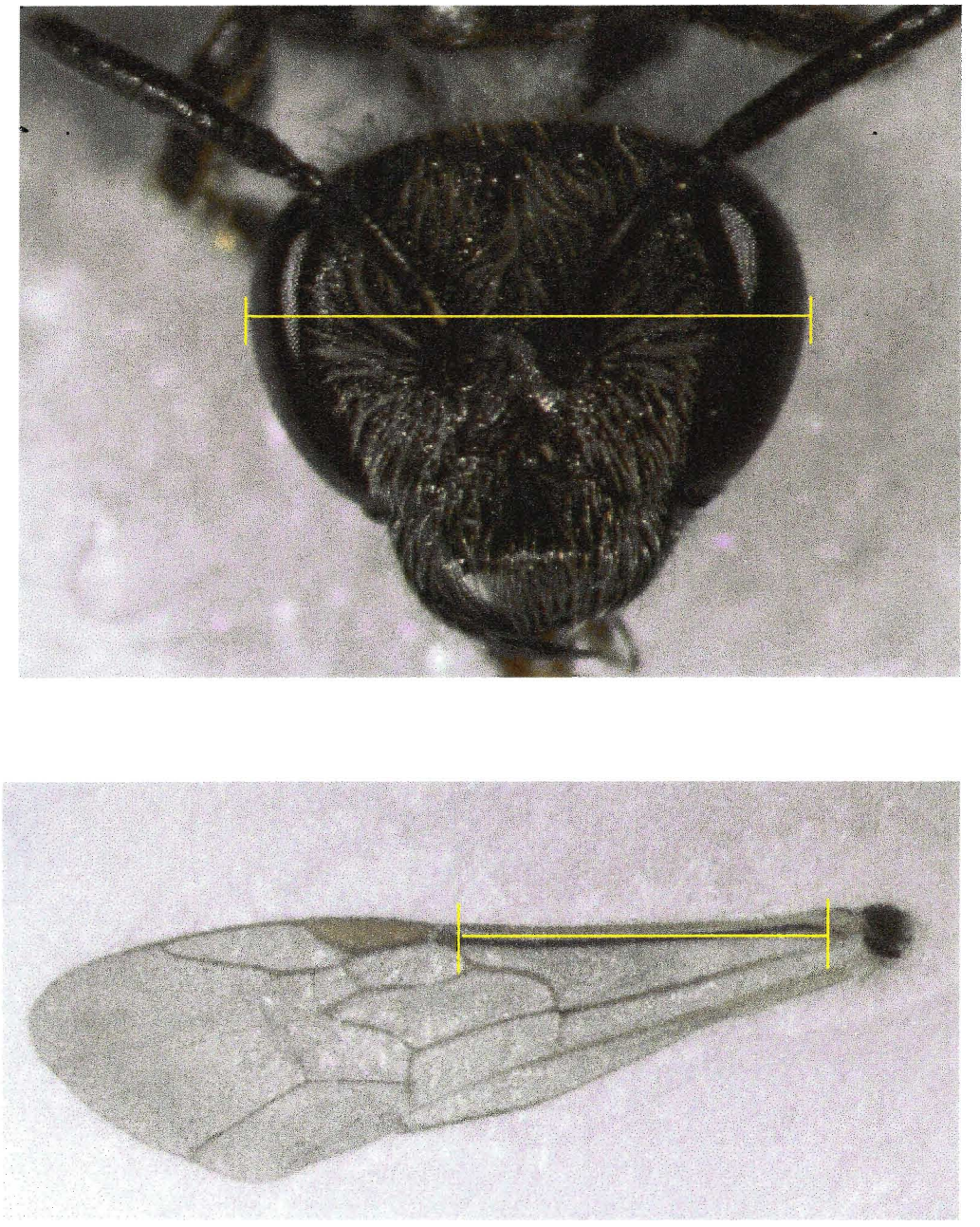

Figure 1.7: Head width and wing length measurements. (A) Frontal view of a female head showing head width, represented by the line drawn across the widest part of the head. (B) A female wing showing the costal vein length, indicated by the straight line.

For size dimorphism calculations for each nest, the mean worker head width was used and for the nest with two queens, the mean queen head width was used.

In order to establish the relationship between mass and head width, dry weight was measured from 29 individuals including 4 adult workers (when captured), 12 female imagos (5 workers and 7 gynes), and 13 male imagos. Specimens were placed in a drying oven at 60^0C for four days and then kept in a desiccator until weighed on a Mettler H2O® analytical balance accurate to 0.0001 gram. After initial measurements, all specimens were placed in the drying oven for another four days and then weighed a second time. Dry weight (mg) for the 29 specimens were plotted against their corresponding head widths and a regression equation calculated using SAS® and Microsoft Excel®: males, $y=7.309x - 9.7994$; and females, $y=14.765x - 27.452$. These equations were used to estimate the dry weight of all individuals for which head widths were known.

Statistical analyses

Minitab®, SAS® and Microsoft Excel® were used to perform statistical analyses. Parametric tests (Analysis of Variance (ANOVA) and Analysis of Co-variance (ANCOVA) were used for all normally distributed data. Non-parametric tests (Kruskal-Wallis) were used for all non-normally distributed data. A Bonferroni (Dunn) T-test was used in conjunction with parametric and non-parametric tests for pair-wise comparisons. Alpha levels for all tests were at the $P=0.05$ level.

Results

Section 1: Nest proximity

Nearest-neighbour distance at MALA1 (mean = 49.5±118 cm, n=75) was significantly greater than the mean distance between nests at ANM (14.8±11.3 cm, n=157; ANOVA, $F=13.18$, $df=1, 231$, $p<0.0001$).

Section 2: Nest Phenology and Brood Development

Figure 1.8 summarizes the phenology and life cycle of *L. malachurum* based on both ANM and MALA1 aggregations in 2000. Brood 1 developed and emerged in early to mid-April and foraged until late May or June (Richards 2000). Based on nests excavated between 15-29 May, Brood 2 developed from mid- to late May, and foraged from early June to late June or July. The production of Brood 2 was approximately 3 weeks long. Based on nests excavated 8-25 June, Brood 3 developed and emerged from early June to late June, and foraged from mid- to late June through to July or August. The production of Brood 3 was approximately 3- 4 weeks long. In southern Greece, *L. malachurum* had a minimum of three broods; it was not known if a fourth brood was produced.

Following the end of foraging activity, queens and workers entered a quiescent or inactive period, which corresponded with the period of brood development. Quiescent periods were observed prior to the emergence of Brood 2 (week 3 at ANM, and weeks 3 and 4 at MALA1) and Brood 3 (week 6) offspring, and lasted approximately 1 to 2 1/2 weeks. Therefore *L. malachurum* ANM and MALA1 aggregations had discrete brood production.

During weeks 6 and 7, I observed the flying behaviours of foragers leaving and entering nests at both aggregations. The foragers at MALA1, observed at approximately 10 am on 7 June, exhibited a delay in their departure and upon exit, flew very rapidly around a large area. They would perform a zigzag pattern over their nest entrance, presumably

Early March	**Nest Initiation:** Queen establishes nest
Late March - Early April	**Initiation of BROOD 1:** Queen forages for Brood 1 (Nests 10 – 14 cm deep)
Early – Mid April	**Quiescent period**
Mid – Late April	**BROOD 1:** Workers emerge
Week 1 7 – 13 May	**Initiation of BROOD 2:** Queen lays eggs, Brood 1 workers forage (Nests 25 – 35 cm deep)
Week 2 14 – 20 May	Brood 1 workers continue to forage
Week 3 21 – 27 May	**Quiescent period** (**ANM:**15-26 May, **MALA1**: 21–28 May No activity outside the nests
Week 4 28 May – 3 June	**Cessation of Brood 2 & Initiation of BROOD 3:** Brood 2 workers emerge Surviving Broods 1 & 2 workers forage for Brood 3 (Nests 55-94 cm deep)
Week 5 4 – 10 June	Brood 1 and Brood 2 workers forage
Week 6 11 – 17 June	**Quiescent period**
Week 7 18 – 25 June	**Cessation of Brood 3 & Initiation of Brood 4:** Workers, males and gynes emerge Workers forage for Brood 4 (Nests >94 cm deep)

Figure 1.8: Colony phenology of *L. (E) malachurum* at Agios Nikolaos Monemvasias, Greece, based on Richards (2000) and this study. The depth at which the brood was found during nest excavations is shown in brackets. In 2000, field studies ended 25 June and so it was not certain whether or not a fourth brood was produced.

orientating themselves by noting the topographic surroundings with respect to their nest entrance (Knerer 1992). When returning with a pollen load, these foragers took a long time (in one instance it took 2 minutes) to enter, usually sitting on a nearby rock and would then fly to and around the nest entrance several times before entering. These behaviours are suggestive of inexperienced bees (Knerer 1992). In contrast, foragers at ANM (observed on 8 June, 7 am) left their nest without hesitation and flew directly off to forage without the orientation flight. When returning with a pollen load, they flew directly into their nest, behaviour that suggested that these bees were experienced (Knerer 1992).

Brood sizes were determined based on nests that were fully excavated during week 2 (ANM and MALA1) and week 4 (ANM) (Fig.1.9). The mean brood size of nests excavated during week 2 at MALA1 was 32.5±17.7 (n=2) and at ANM was 34.0±2.6 (n=3). Data for the two sites were combined giving a mean Brood 2 size of 33.4±9.1 (n=5).

Brood 3 production began during week 5 and ended during week 7 (Fig. 1.9). Nests excavated during week 6 contained all stages from pollen balls to pupae and therefore best represented Brood 3. The mean size of Brood 3 was 48.3±13.3 (n=4), which was not significantly larger than the mean size of Brood 2 (Kruskal-Wallis $F=3.99$, $df=1,8$, $p<0.0860$), albeit sample sizes were small.

Figure 1.10 shows the ratios of male and female brood and those of unknown sex each week, based on the numbers of sexable and non-sexable pupae and larvae (including parasites occupying brood cells) from fully excavated nests. Male brood were first found in nests excavated during week 5 (4 –11 June), and so were first produced in Brood 3. Male brood were also found in nests that were excavated during weeks 6 and 7, indicating that male production lasts a minimum of 3 weeks. One nest (ANM426), partially excavated during week 7, had 2 adult males in the nest tunnel suggesting that adult males emerge by week 7. Size measurements of female brood (Section 3) reveal that gyne production began

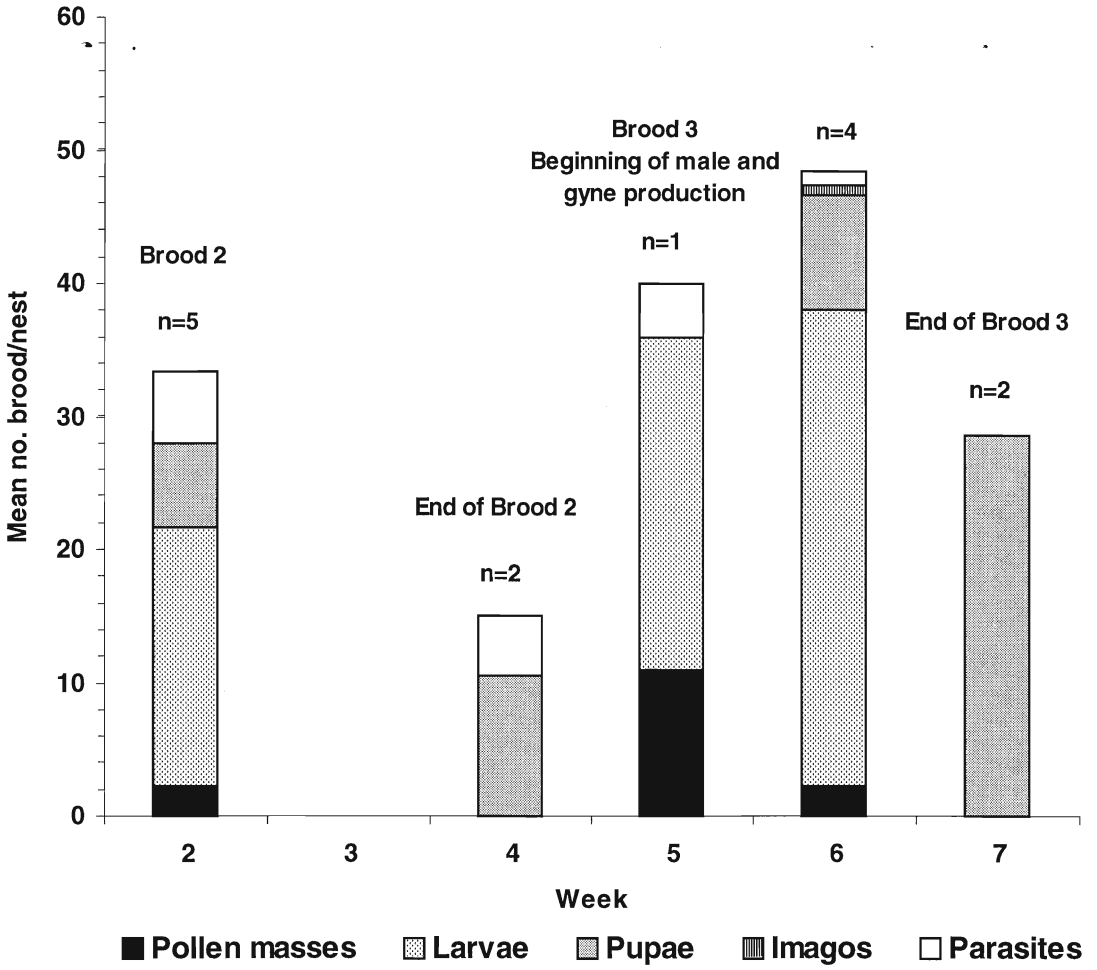

Figure 1.9: Brood development of *L. malachurum*. No nests were excavated during week 3. The two nests from week 7 were only partially excavated. The numbers above the bars represent the numbers of nests excavated each week. The category "Parasites" includes *L. malachurum* larvae with parasites attached and larval parasites that were the sole occupants of their brood cells.

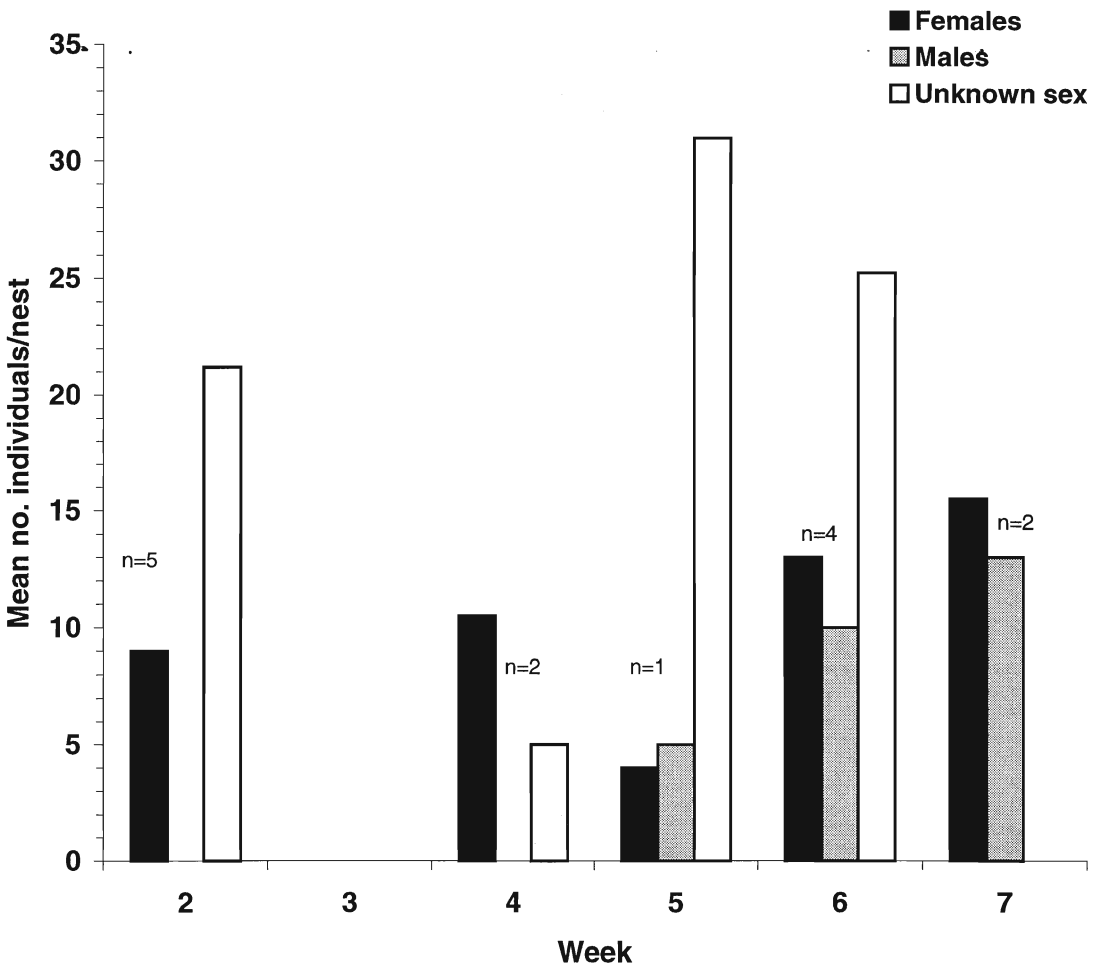

Figure 1.10: Average numbers of brood per week. Numbers of males and females are based on counts of sexable pupae. Unknown sex refers to brood that were too young to be raised to pupation outside their brood cells and so could not be sexed. Unknowns also include parasite larvae that occupied brood cells. Numbers above the bars were the numbers of nests excavated. Data for week 7 were from partially excavated nests and were included here to represent an approximate sex ratio for that week.

during week 5 (4 – 10 June), the beginning of Brood 3 (Fig. 1.9).

Figure 1.11 shows the age distributions of Brood 3 worker, gyne, and male brood and unknown caste from nests excavated during weeks 5, 6, and 7. The ages of brood were grouped from 1 (larval stages: small larvae to prepupae), 2 (young pupal stages: white pupae to black pupae) and 3 (old pupal stages: ¼ pigmented pupae to imagos) for statistical analyses. When workers and gynes were combined for comparisons with males, males were significantly older than females (Likelihood χ^2= 11.758, df =2, p<0.003) suggesting that Brood 3 was protandrous (males emerge prior to females).

Table 1.2 shows the numbers of worker, gyne and male brood from nests excavated each week. Eighteen brood, ranging in size from fully-grown larvae to the pre-pupal stage, were assumed to be gynes because they were significantly larger than adult males and workers from the same nest. Fifteen gyne pupae (five week 6 and ten week 7) had heads too damaged to measure, but were assigned to this caste based on comparisons of their body size to those females of known caste type. Accordingly, sex ratios were calculated by comparing: (1) numbers of females (gynes and workers) to males, and (2) numbers of gynes to males. The numbers of workers and gynes were combined for weeks 2 and 4 to represent Brood 2 and for weeks 5, 6 and 7 to represent Brood 3 females. Week 7 nests were included to represent approximate sex ratios. All Brood 2 offspring were workers (female). Caste assignment of 87 female progeny revealed that a minimum 45% (71 of 158 males and females) of Brood 3 progeny were gynes. The overall sex ratio (♀:♂) of Brood 3 (weeks 5-7) was 1.2:1 (87/71), which was not statistically different from a 1:1 ratio (Likelihood χ^2 =0.813, df =1, n.s.; excluding week 7: Likelihood χ^2= 0.601, df=1, n.s.). Excluding workers, a ratio of 1:1 gynes to males (71/71) was recorded.

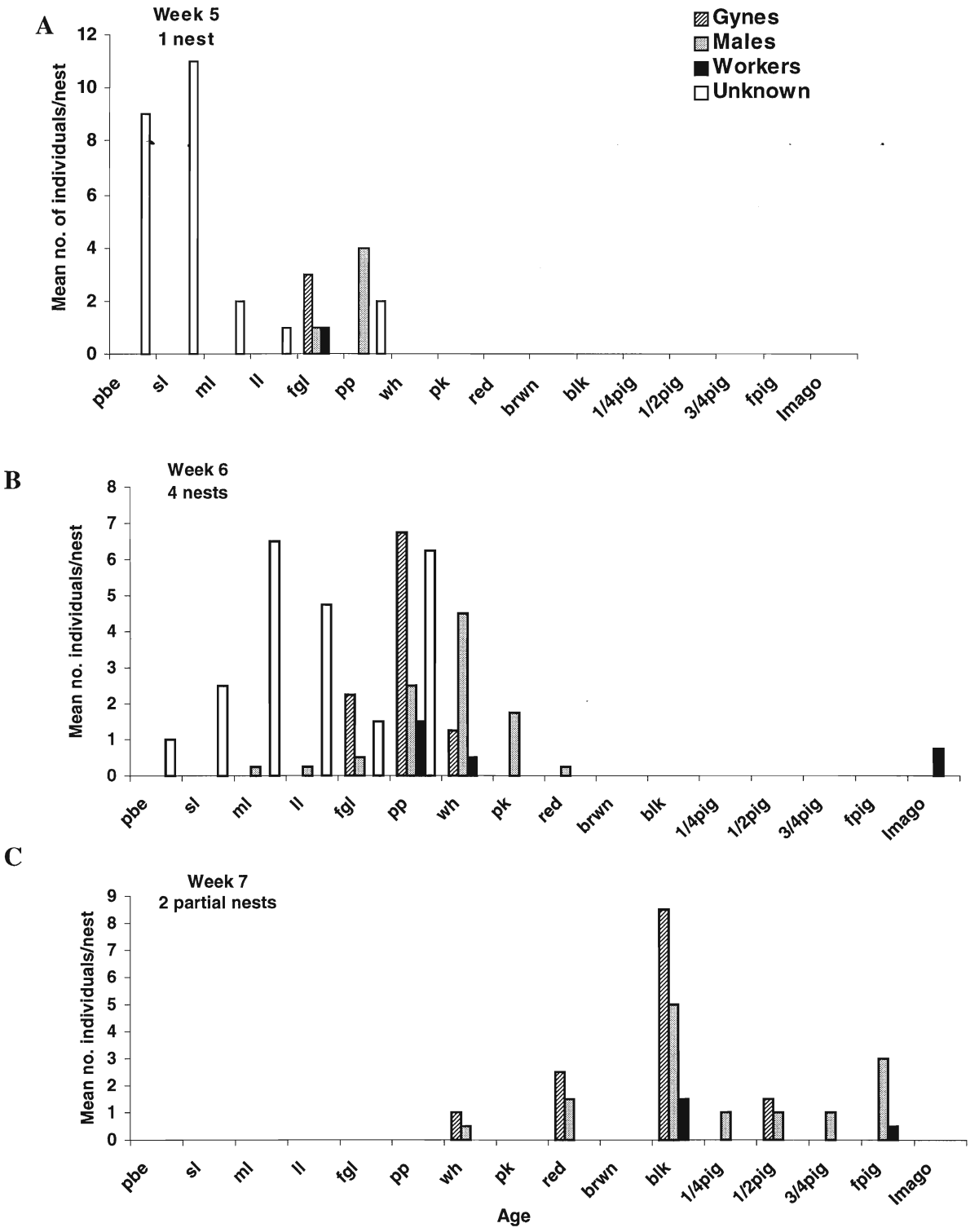

Figure 1.11: The timing of worker, male and gyne production, based on the age distribution of offspring for weeks 5-7. Unknown sex is also shown.

Table 1.2: The numbers of female (gynes and workers), and male brood and unsexed larvae from nests excavated from weeks 2-7. Unsexed brood were mostly larvae too young to raise to pupation.

Brood	Week (No. nests)	Workers	Gynes	Total females	Males	Unsexed brood (%)
2	**2** (5)	45	0	**45**	**0**	**95** (68)
	4 (2)	21	0	**21**	**0**	**0** (0)
	Totals	66	0	**66**	**0**	**95** (59)
3	**5** (1)	1	3	**4**	**5**	**27** (75)
	6 (4)	11	41 (5*)	**52**	**40**	**90** (49)
	7 (2)	4	27 (10*)	**31**	**26**	**0** (0)
	Totals	16	71	**87**	**71**	**117** (43)

* Numbers in brackets represent the number of female pupae without head width measurements and to whom the caste type 'gyne' was assigned.

Parasites

Parasites, such as mutillids and *Sphecodes*, were observed crawling or flying around nest entrances, and bombyliid larvae were found within brood cells, at both aggregations during all weeks of the study period. In one instance (16 May), a mutillid was caught crawling out of nest MALA76 with *L. malachurum* body parts. On 15 June (week 6) many mutillids were observed crawling around the ANM aggregation and two were seen entering a nest. Little to no *L. malachurum* activity was observed from the nests at this time.

Figure 1.9 shows the mean number of parasites per week. Of 20 nests (8 partially and 12 fully excavated) at both aggregations, 13 (65%) of the nests had brood cells containing parasites, parasitized *L. malachurum* larvae, adult mutillids, or a combination of the three. Table A1 (Appendix A) shows the numbers of parasites per nest for weeks 2-7.

Queens

Nests excavated at MALA1 and ANM were combined to determine the mean number of queens per nest each week (Table 1.3). A queen was captured from one nest (ANM NA1) during week 6 and from each of two other nests in week 7 (ANM N426 and N427) that were only partially dug, and therefore may only represent a minimum number of queens in these nests. One nest (ANM N378) excavated during week 6 had two queens present, increasing the mean number of queens per nest to 1.3 for week 6. These individuals were both considered queens because they were the two largest females in the nest, had the same severity of wear and tear, were both mated, along with having the same degree of ovarian development (Sections 3, 5 and 6).

Queens were found in nests excavated during weeks 2, 4, 6 and 7. Two queens were found alive in nests excavated on 21 June (week 7), when some of Brood 3 progeny had emerged. Thus, the minimum life span of queens after the overwintering period, at the ANM aggregation, was approximately 16 weeks (from March – June).

Table 1.3: Average number of queens per nest at ANM and MALA1. Full and partial indicate which nests were fully or only partially excavated.

Week		Total nests excavated	No. nests with queen(s) (%)	Queens per nest (average)
2		5	4 (80)	0.8
4		2	1 (50)	0.5
5		1	0 (0)	0.0
6	**partial**	1	1 (100)	1.0
	full	3	3 (100)	1.3*
7	**partial**	2	2 (100)	1.0

*Calculated based on 4 queens divided by 3 nests (N378 had two queens)

Workers

I used different methods to determine the number of *L. malachurum* workers per nest at MALA1 and ANM, and for this reason, the two aggregations were considered separately.

MALA1 aggregation

Field observations at MALA1 commenced on 10 May. At that time, workers from Brood 1 were seen foraging. The number of workers per nest was estimated based on counts of foragers and guards observed at each nest (week 1) and foragers that were collected as they left the nest (weeks 2, 5, 7) (Table 1.4). Foragers included workers observed or caught arriving at or departing from the nest and guards were workers that remained at the nest entrance. Foragers were not collected during quiescent periods (weeks 3, 4, 6).

There was a significant positive correlation between the mean number of workers with weeks 1, 2, 5, 7 (Pearson correlation $\rho=0.64655$, $p<0.0001$, $n=37$; mean number of workers in week one: 2.4±1.7, n=25; mean week two: 4.2±2.1, n=9; mean week five: 4.5±2.1, n=2; week seven: 13, n=1). Each week at MALA1 the proportion of nests with guards was estimated during ≥ 30 minutes of nest observations or during collection experiments. During 13 surveys, between 11 May and 24 June, 52% (80/155) of nests had a guard (Tables 1.4, A2 Appendix A).

ANM aggregation

At ANM, means of workers produced in Broods 1-3 were based on fully excavated nests (Table 1.5). Week 4 represented the end of Brood 2. At this time, nests may have included old Brood 1 workers and new Brood 2 workers, some of the latter were still in the pupal stage.

Table 1.4: The minimum and mean number of workers (foragers + guards) per nest, as well as the proportion of nests with guards at MALA1. Data were based on observations of nests for ≥ 30 minutes or collection of foragers leaving the nest.

Week	Nests (n)	No. Workers		Nests with guard	
		Minimum	Mean ± s.d.	(n)	(%)
1	25	0	2.4±1.7	17	(68)
2	9	1	4.2±2.1	4	(44)
5	2	3	4.5	2	(100)
7	1	13	13.0	1	(100)

Table 1.5: The mean numbers of workers and foragers found in fully excavated nests at ANM in Broods 1-3. The mean number of Brood 1 workers was based on counts of old adult workers in nests excavated during weeks 2 and 4. In Brood 2, it was based on new adult workers and worker pupae (i.e. brood), and worker pupae only in Brood 3.

Brood	Nest	Week	No. live worker brood	No. adult workers
1	N386	2	-	5
	N388	2	-	3
	N390	2	-	6
	N382	4	-	2
	N385	4	-	7
	Mean no. workers/nest		-	**4.6±2.1**
2	N386	2	23	-
	N388	2	18	-
	N390	2	34	-
	N382	4	13	3
	N385	4	8	5
	Mean no. workers/nest		**-**	**20.8±8.2**‡
3	N379	5	1 (27*)	13†
	N376	6	2 (36*)	9†
	N377	6	1 (16*)	10†
	N378	6	4 (25*)	11†
	B1	6	4 (20*)	0
	Mean no. workers/nest		**2.4±1.5**	**-**

‡ The mean for Brood 2 workers calculation included adding the post-emergence Brood 2 workers to worker pupae from nests ANM382 and ANM385.

* The numbers in brackets represent the number of brood that were still in larval stages for which sex or caste could not be determined and so some of these individuals may have been workers.

† The numbers of foragers found during week 3, but may have been produced in either brood 1 or brood 2. The calculation of the mean did not include these numbers.

In order to determine which adult workers were new Brood 2 workers during week 4, total wear and ovarian development scores (OD) were assessed (see Sections 5 and 6). Workers that had total wear scores ≤ 1 along with ovarian development scores of 0 or 0.1 were considered new workers. Based on these criteria, 60% (3/5) of workers from nest ANM382 and 42% (5/12) from nest ANM385 had total wear scores ≤1 and OD scores of 0 or 0.1. These eight workers were included in counts of Brood 2 workers, while the other nine were considered Brood 1 workers. During weeks 5-7 there were 55 adult workers caught from fully excavated nests that may have been produced in either Brood 1 or 2 and so were not included in counts for either Brood 1 or 2.

The mean number of workers in Brood 2 (20.8±8.2, n=5) was significantly larger than the mean number of workers in Brood 1 (4.6±2.1, n=5) and Brood 3 (2.4±1.5, n=5) (Kruskal-Wallis F= 25.78, df=2, 16, $p<0.0001$; Bonferroni (Dunn) $T_{brood}=2.71776$, MSE=21.39, df=14, $p<0.05$).

Section 3: Caste characteristics

Fat stores as an identification of caste

In order to determine whether or not only gynes emerge with abdomens full of fat bodies, bee abdomens were dissected. Dissections of newly emerged gynes (n=3), workers (n=6) and males (n=2) revealed that all imagos had abdomens full of fat stores. Furthermore, sixteen adult workers that were caught leaving the nest had abdomens full of fat stores. Fifteen of these were from nests excavated during weeks 2 and 4 (Brood 2), the other was caught from a nest excavated during week 7, the end of Brood 3. To determine if these individuals were newly emerged, ovarian development and wear and tear were assessed (see Sections 5 and 6). All had ovarian development scores of 0 or 0.1 and 88% (14/16) had mandibular wear scores of 0 or 1, suggesting that these individuals were newly

emerged or young. The other two females had mandibular wear scores of 2 (week 4) and 4 (week 7).

Body size

Queens, adult workers, and female and male brood were compared based on head width (HW) and wing length (WL) measurements (Fig. 1.12a). Head width and wing length were positively correlated (Pearson Correlation ρ =0.87226, $p<0.0001$, n = 370 for all castes and males). Head widths were used for all statistical analyses because wing lengths of pupae could not be measured. Queen head width (2.46±0.09 mm, n=14) was not significantly different than that of gynes (2.48±0.04 mm, n= 40), but queens and gynes were both significantly larger than workers (2.19±0.07 mm, n=388) (Kruskal-Wallis F=115.56, df = 2, 309, $p<0.0001$, Bonferroni (Dunn) T_{Caste} = 2.40717, MSE=4529.43, df=307, $p<0.05$). Two queens (both from the nest ANM378) had head widths of 2.60 mm and 2.54 mm.

Head width of Brood 1 workers (2.19±0.06, n=159) was not significantly larger than that of Brood 2 workers (2.18±0.10, n=82), but both were significantly smaller than Brood 3 workers (2.28±0.08, n=15) head width (Kruskal-Wallis F=7.12 df=2, 255, $p<0.0010$, Bonferroni (Dunn) T_{Brood} = 2.4100, MSE=5067.92, df=253, $p<0.05$). The mean head width of 132 adult workers caught during weeks 5-7 was 2.12±0.06, but these individuals could not be assigned to any Brood. The mean head width of males was 1.94±0.09 mm, n=55.

Based on head width measurements, queens (n=14) were 10.6% larger than workers (n=388); size differences ranged from 5.4% to 14% in excavated nests (Table A3, Appendix A). Based on wing lengths, queens (n=14) were 11.8% larger than workers (n=322); size differences ranged from 6.5% to 17.9% in excavated nests.

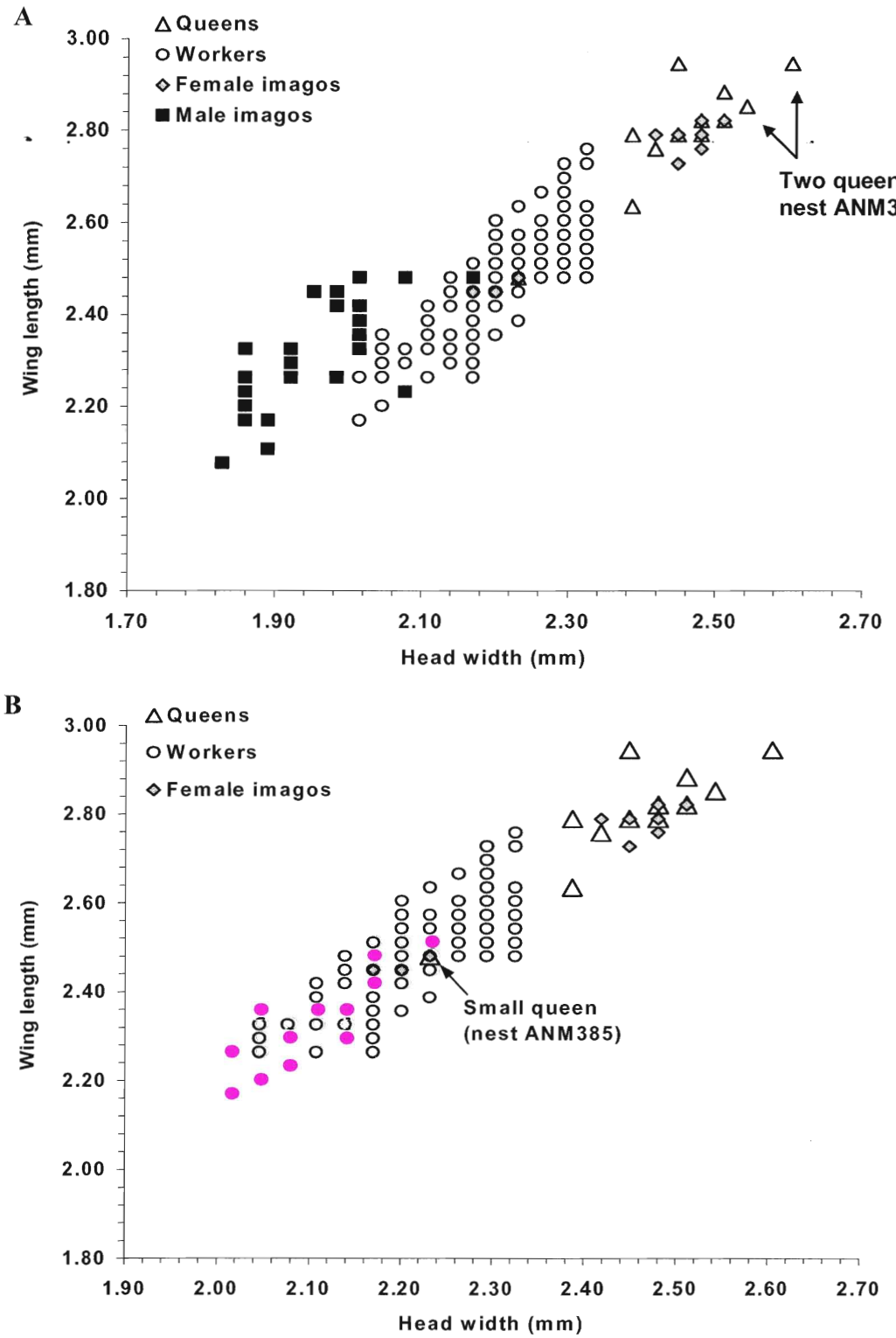

Figure 1.12: Size distributions (A) Distribution of queens (n=14), adult workers (n=318), and female (n=15) and male (n=27) imagos based on wing length and head width measurements. (B) Distribution showing the small queen from nest ANM385 along with the adult workers found in the same nest (pink circles).

There was an overlap between worker and queen size based on head widths only because one queen (nest ANM385) that had a head width of 2.23 mm (Fig.1.12). The other thirteen queens had head widths ≥ 2.39 mm, all gynes had head widths ≥ 2.42 mm, and all adult workers had head widths ≤ 2.36 mm. The small queen had a high degree of ovarian development, mandibular wear and wing wear. She was also the largest female in the nest and was mated at a time when no males were present.

Fifteen female brood had measurable head widths and wing lengths; 4 individuals had head widths ≤ 2.36 mm and the other eleven were ≥ 2.42 mm. To determine when females of each size class were produced, the distribution of all females with measurable head widths was plotted each week (Fig. 1.13). All female brood, produced during weeks 2 and 4 (Brood 2), were ≤ 2.36 mm, indicating that all Brood 2 offspring were workers. During weeks 5-7, however, female head widths were both above and below 2.36 mm, indicating that Brood 3 contained workers and gynes, confirming the commencement of gyne production.

In order to determine if queen size influenced their developing brood, the size relationship between queens and their offspring was investigated (Fig. 1.14). There was no correlation between queen head width and the per nest averages of Brood 1 worker head widths (Pearson Correlation ρ =0.60219, n.s., n=6), Brood 2 worker head widths (Pearson Correlation ρ = 0.53705, n.s., n=10), or Brood 3 worker head widths (Pearson Correlation ρ = -0.02124, n.s., n=5).

Section 4: Investment ratios based on dry weight measurements

In order to establish the investment ratios for each caste and sex, I measured the dry-weights of adult workers (n=4), newly emerged females (n=12) and newly emerged males (n=13). Figure 1.15 shows the relationship between the measured head widths and dry

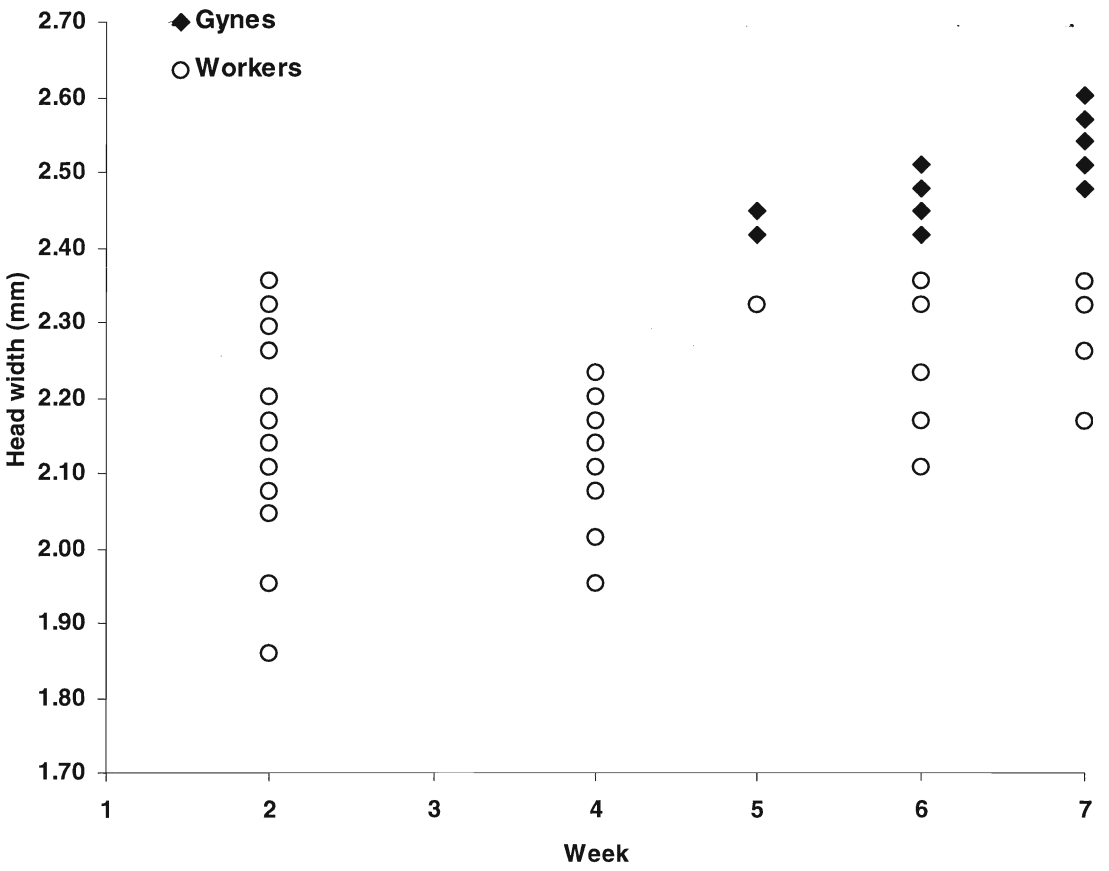

Figure 1.13: Worker and gyne brood head widths each week. Workers had head widths less than or equal to 2.36 mm and were produced in all weeks. All gynes had head widths greater than or equal to 2.42 mm and were produced only during weeks 5-7 (Brood 3).

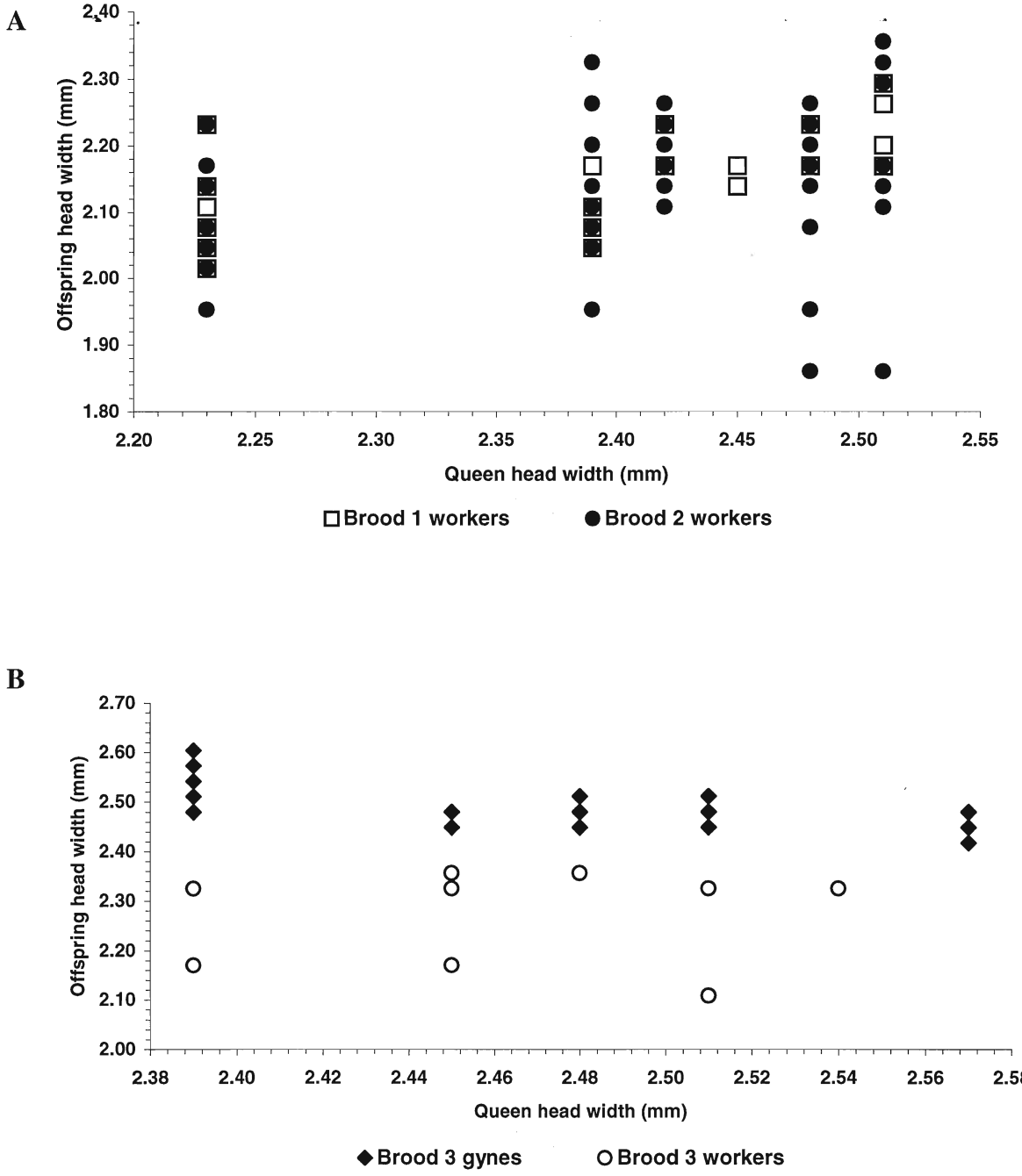

Figure 1.14: Influence of queen head width on head widths of brood in the same nest. (A) Relationship between queen head width and worker head width in Broods 1 and 2. (B) Relationships between queen head width and daughter head width in Brood 3.

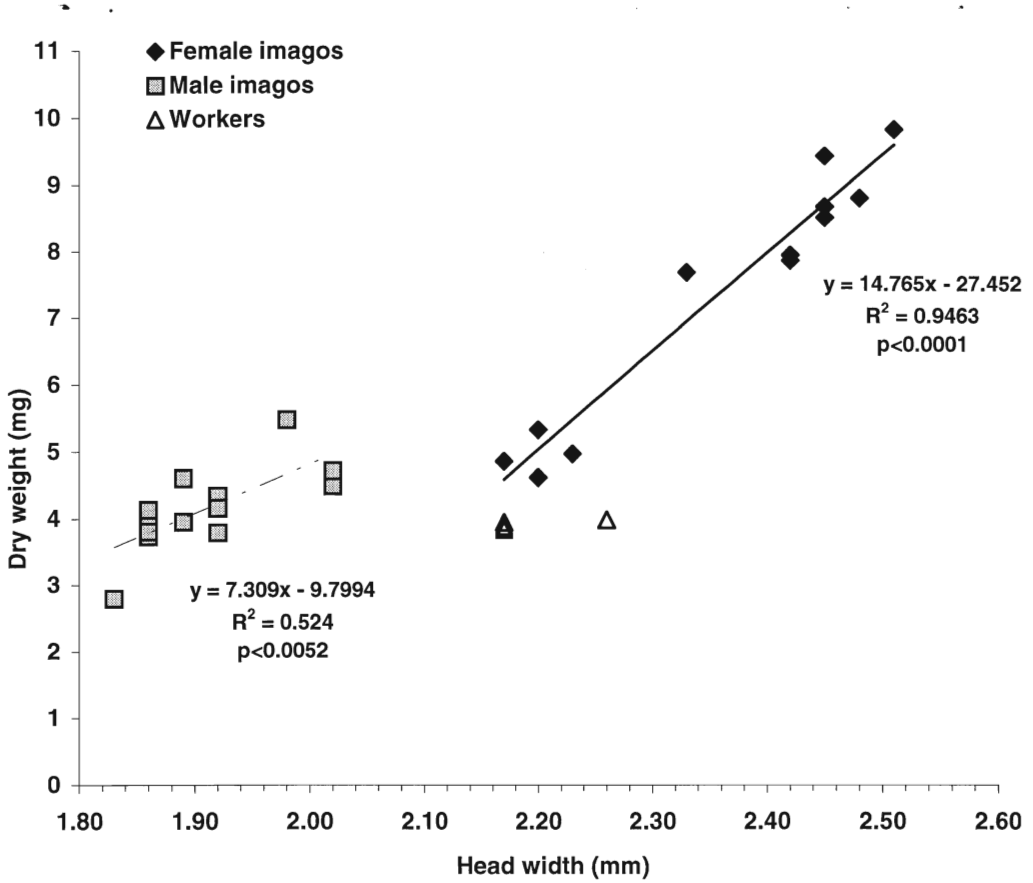

Figure 1.15: Relationship between head width and dry weight in newly emerged male and female brood, as well as lines of best fit, the corresponding R^2 values and p-values are shown for the newly emerged males and females.

weights for the 29 individuals. This relationship was used to estimate dry weights at emergence from head width and to calculate the investment ratios for gynes to males for excavated nests (Table 1.6). The mean weight of Brood 1 workers was 4.89±0.90 mg, n=159, Brood 2 workers was 4.73±1.49 mg, n=82 and Brood 3 workers was 6.15±1.24 mg, n=15. The mean weight of workers that may have been produced in either Brood 1 or Brood 2 was 5.18±0.87 mg, n=132. The overall mean weight of workers was 5.00±1.10 mg, n=388. The mean weight of males and gynes was 4.44±0.68 mg, n=55 and 9.18±0.60 mg, n=40, respectively. The overall investment ratio of gynes to males was 1.4:1.

Section 5: Reproductive characteristics of Queens and Workers

A total of 14 queens collected from nests excavated at MALA1 and ANM were dissected and assessed for matedness and degree of ovarian development (Fig. 1.16). All, queens assessed (except one) were mated and none had an ovarian score of zero (matedness could not be determined for one queen because her abdomen was too damaged). Twelve of 14 queens (86%) had ovarian development scores of at least 0.5, the highest score being 5 (nest MALA171 found during week 2). The other two queens had scores of 0.1 because their ovaries had degraded. The mean ovarian score of queens was 1.68±1.5, n=14. The two queens from the same nest were mated and both had total ovarian scores of 1.0.

Matedness could be determined for 278 workers, of which only 2 (0.7%) had spermathecae full of sperm. These two workers were from nest ANM426; a nest excavated during week 7, the end of Brood 3. A total of 46 (15.5%, n= 297) workers had no ovarian development and 78 (26%, n=297) of workers had a total ovarian score of 0.1 (ovaries containing nurse cells, small oocytes, or ovaries that were slightly thickened). Therefore, 58% of workers had ovarian development greater than 0.1 (i.e. ovarian scores ≥ 0.25). None of the mated workers had well-developed ovaries. The mean ovarian development score of

Table 1.6: Investment ratios (gynes to males) for excavated nests at ANM during Brood 3. The total weights for gynes and males are shown for each nest; the numbers in parentheses are the numbers of gynes or males. The investment ratios were calculated using the total weight of gynes divided by the total weight of males.

Nest	Gynes		Males		Investment ratio (gyne/male)
	Mass (mg)	(n)	Mass (mg)	(n)	
376	9.2	(1)	31.7	(8)	0.3 : 1
377	27.1	(3)	14.1	(4)	1.9 : 1
378	78.9	(9)	34.8	(8)	2.3 : 1
379	17.0	(2)	22.1	(5)	0.8 : 1
B1	45.4	(5)	25.3	(6)	1.8 : 1
426*	46.3	(5)	58.6	(12)	0.8 : 1
427*	116.6	(12)	53.2	(11)	2.2 : 1
Total	**340.4**	**(37)**	**239.7**	**(54)**	**1.4 : 1**

* Nests ANM427 and ANM426 (partial excavations) are included to show approximate investment ratios for week 7.

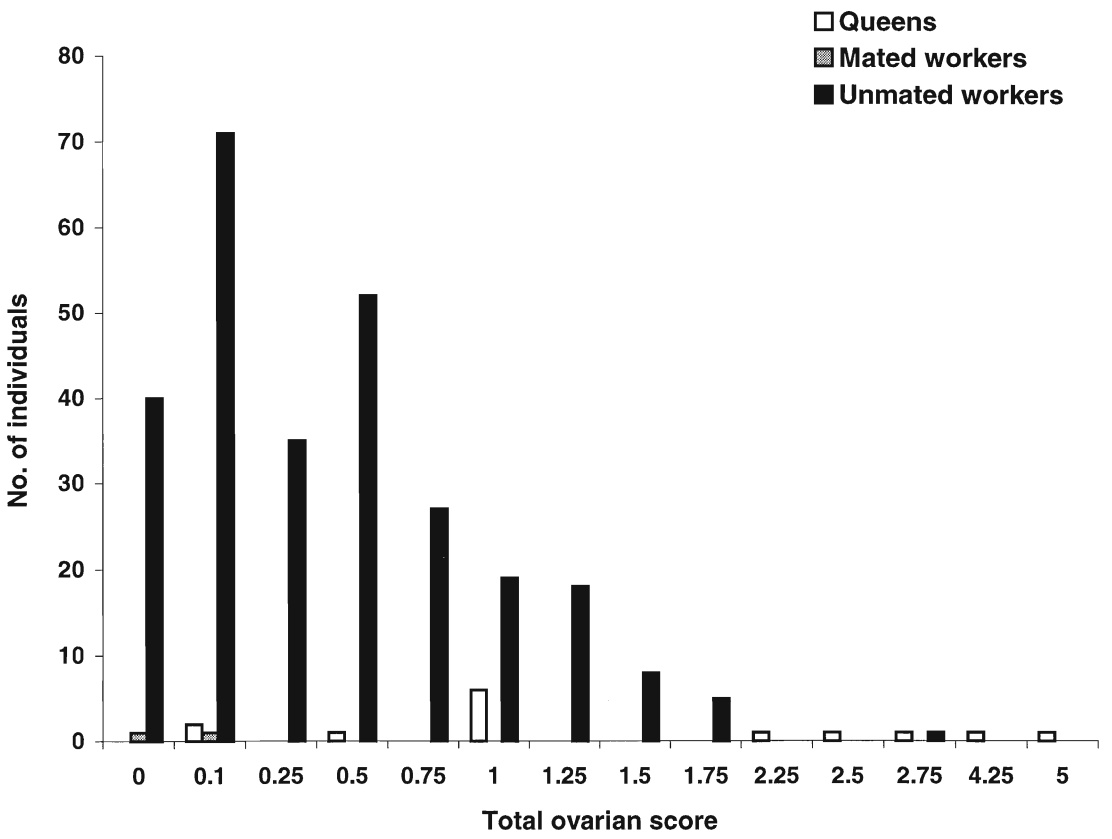

Figure 1.16: Total ovarian scores for queens and mated and unmated workers at ANM and MALA1. A score of 0 represents no ovarian development. A score of 0.1 represents ovaries that had nurse cells or oocytes that were too small to score, or ovaries that were slightly thickened. Total scores were derived by summing individual oocyte development where 0.25 = ¼ developed, 0.50 = ½ developed, and so on.

workers was 0.50±0.48, n=270. An ovarian development score of 2.75, the highest ovarian development score for a worker, was observed in one worker caught from nest ANM339 during week 4 (Brood 2).

If queen control over her worker daughters was related to body size (i.e. the larger the size differential, the better the queen maintained reproductive control), then it was predicted that larger workers may have had more opportunities to lay their own eggs. Young workers were identified by, and excluded from analyses if they had, ovarian development scores of 0 or 0.1 along with mandibular wear scores of 0 or 1, indicating that they were newly emerged. Ovarian development was not normally distributed, but a significant positive correlation between the degree of ovarian development and head width among workers existed (Pearson correlation $\rho = 0.12137$, $p < 0.0484$, n=265) (Fig. 1.17). There was no significant correlation, however, between the percent size difference between queens and workers and the degree of ovarian development (Pearson correlation $\rho = -0.20626$, n.s., n=50).

If workers laid male eggs, then there should have been a correlation between the onset of male production and an increase in the number of workers with well developed ovaries, and eggs ready to lay (at least 1/2 developed). The onset of male production at ANM occurred in week 5 (4 – 10 June) and continued through to at least the end of week 7 (18 – 25 June). Twenty-eight percent of workers (84/297) had at least one oocyte 1/2 developed. Figure 1.18 shows how many of these 84 workers were found each week. Five-percent of these workers were found during week 2 (n=4/84, mean OD score = 0.88±0.43). Beginning one week prior to (week 4), and during the onset of male production (week 5), 29% (n=24/84, mean OD score = 1.16±0.45) and 43% (n=36/84, mean OD score = 1.06±0.37) of workers had ovarian development suggestive of egg laying. These numbers decreased towards the end of male production:

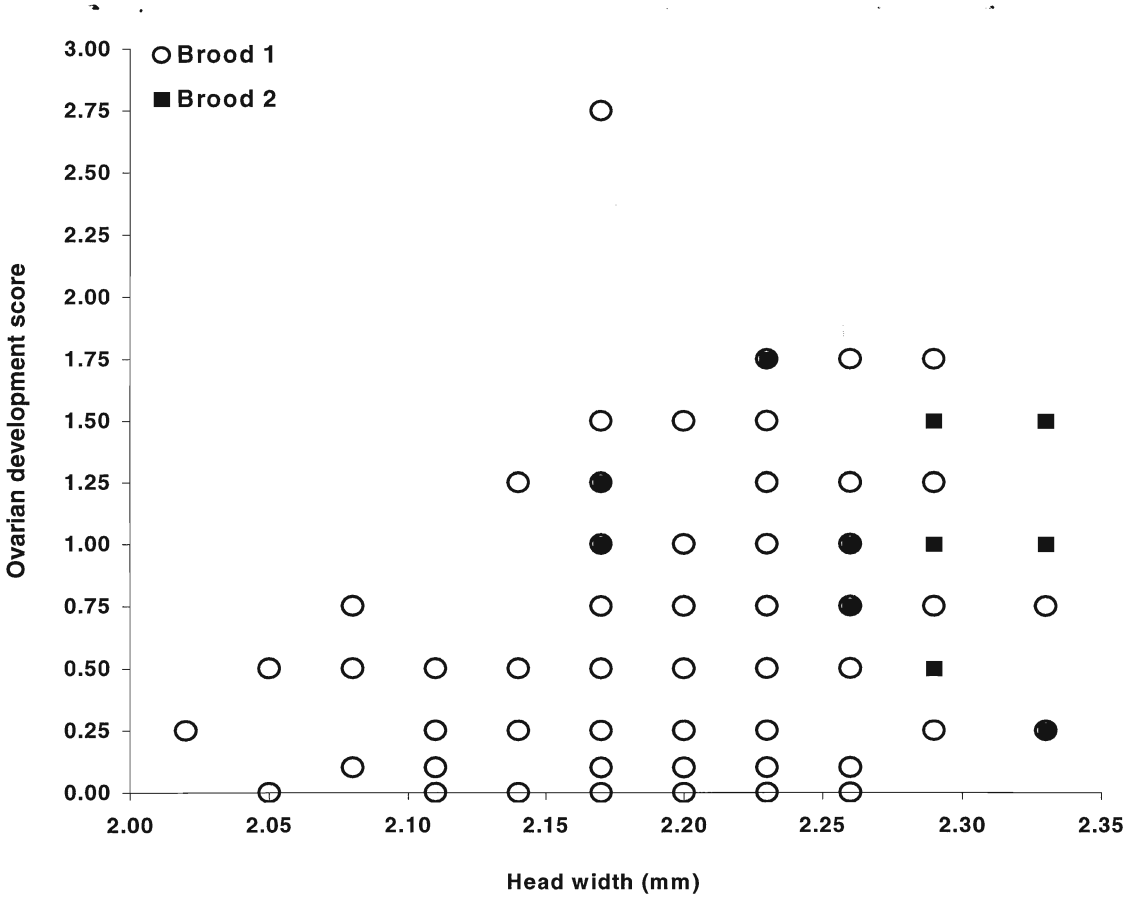

Figure 1.17: Head width and the degree of ovarian development for adult Brood 1 and 2 workers, excluding young workers. Larger workers had higher degrees of ovarian development than smaller workers (statistics in text).

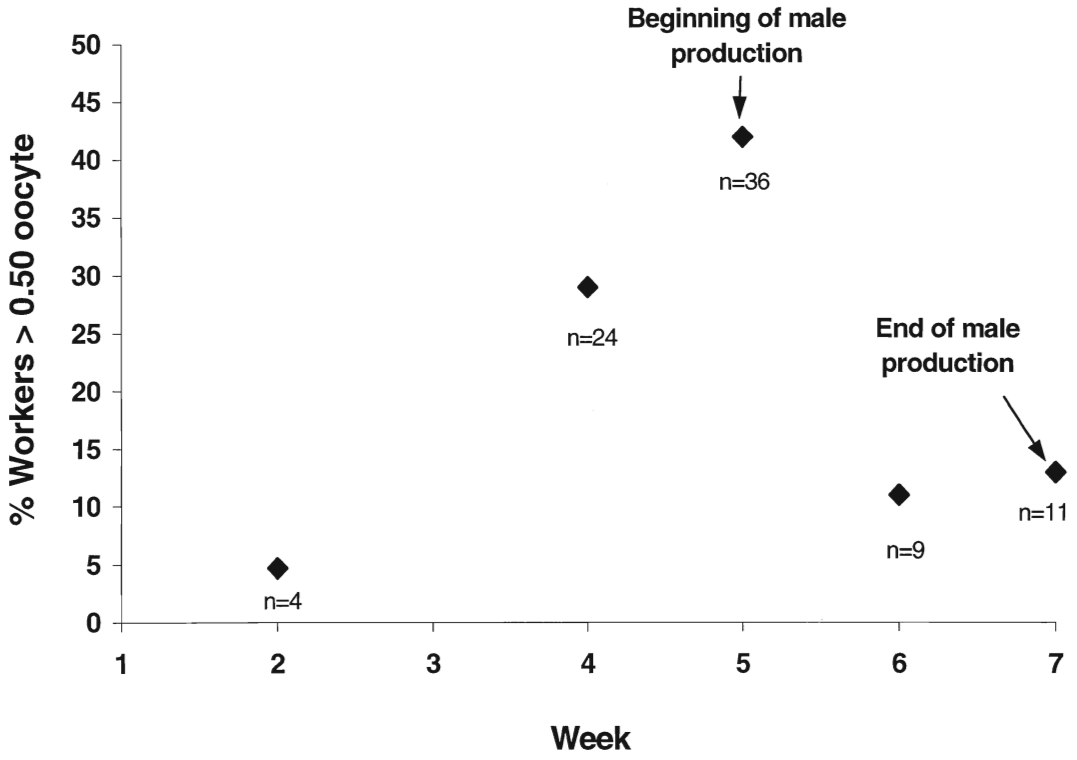

Figure 1.18: The proportion of workers (n=84) with at least one oocyte ≥0.50 each week. Numbers below each point refer to the number of workers. Male production began during week 5 and ended in week 7. Weeks 1-4 = Brood 2 and weeks 5-7 = Brood 3. For specific dates refer to page 41.

week 6, 11% (n=9/84, mean OD score = 0.64±0.18); and week 7, 13% (n=11/84, mean OD score = 1.16±0.30).

Section 6: Wear and tear as a measure of activity

Figure 1.19 shows the distribution of wing wear, mandibular wear and total wear scores of queens and workers. The minimum total wear score of queens was 7. Eleven out of 14 (79%) queens had total wear scores of 9 or 10. There was no correlation between queen wing and mandibular wear (Pearson Correlation $\rho = 0.09337$, n.s., n=14). Queen wing wear, but not mandibular wear, increased significantly each week (Pearson Correlation $\rho_{\text{wing wear}} = 0.59792$, n=14, $p<0.0239$; $\rho_{\text{mandibular wear}} = 0.15791$, n.s., n=14). The two queens from the same nest both had total wear scores of 10.

There was a positive correlation between worker wing and mandibular wear (Pearson Correlation $\rho = 0.23942$, $p<0.0001$, n=276, excluding young workers). Most workers had high mandibular wear scores (MW scores ≥3 = 58%, n=303), and low wing wear scores (WW scores <3 = 95%, n=303). Worker mandibular wear, but not wing wear, scores were significantly higher each week (Pearson Correlation $\rho_{\text{mandibular wear}} = 0.18449$, $p<0.0021$, n=276; $\rho_{\text{wing wear}} = 0.2707$, n.s., n=277). The mean mandibular wear score of workers (2.8±1.2, n=276, excluding young workers, n=27) was significantly less than that of queens (4.6±0.6, n=14, Kruskal-Wallis F=13.10, df=4, 281, $p<0.0001$).

Highly cooperative workers were expected to have a low degree of ovarian development associated with a high degree of mandibular wear. However, there was no correlation between worker ovarian development and mandibular wear (Pearson Correlation $\rho = -0.4275$, n.s., n=270) (Fig. 1.20). Similarly, there was no correlation between mandibular wear and ovarian development for queens (Pearson Correlation $\rho = 0.09160$, n.s., n=14).

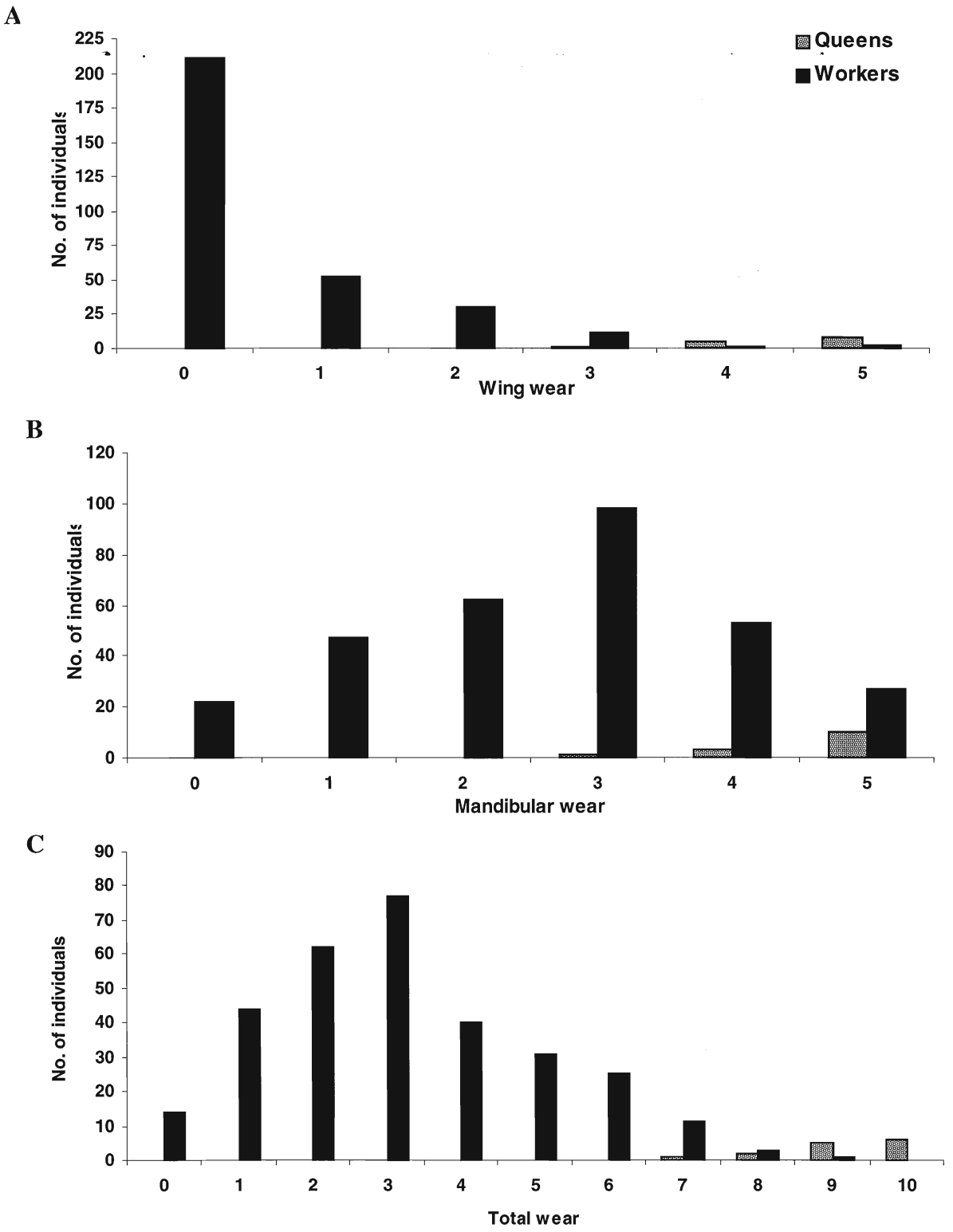

Figure 1.19: Comparison of queen and worker (A) wing wear, (B) mandibular wear and (C) total wear (wing wear + mandibular wear) scores.

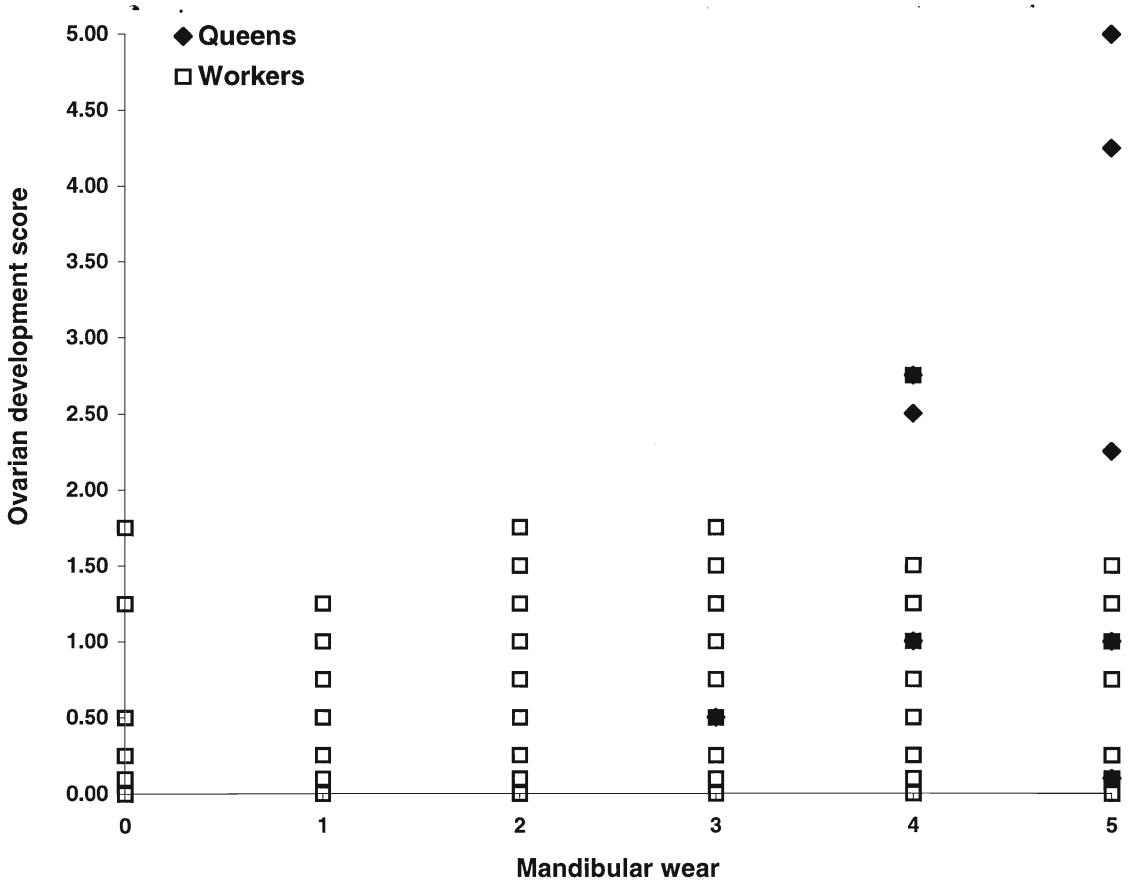

Figure 1.20: The correlation between the degree of mandibular wear and ovarian development for workers (n=270, p-value=0.4843) and queens (n=14, p-value=0.7555). The queens from nests ANMA1 and ANMB1 had regressed ovaries (OD score of 0.1).

Discussion

L. malachurum is an obligately eusocial sweat bee that exemplifies strong eusociality because of a well-defined caste system of queens that are the principal reproductives and workers that are primarily helpful. Intraspecific geographic variation exists, however, and current evidence suggests a north-south cline of decreasing eusociality (Knerer 1992; Richards 2000). The results of this study reveal the colony social organization of a population that is further south than any other *L. malachurum* population studied thus far, and provides evidence in support of a cline of decreasing eusociality, as well as insight into proximate reasons for varying levels of worker altruism.

Colony development

Timing of major colony events

Variation in nesting phenology was observed between the two aggregations, ANM and MALA1. The differences in flying behaviours exhibited by foragers at the two aggregations during weeks 6 and 7 suggested that ANM workers were older than workers at MALA1. Knerer (1992) observed similar behaviours and attributed this difference to variations in the ages of workers. At MALA1, younger Brood 2 workers began to emerge around week 6 and 7 when, at ANM, Brood 3 production had stopped and the developing brood were emerging. Brood 2 development at MALA1, therefore appeared to last approximately 2 ½ weeks longer than at ANM.

The difference in Brood 2 timing could not be attributed to variation in the local climate because the two aggregations were separated by less than a kilometer. Furthermore, the laying of Brood 2 appeared to be in synchrony at ANM and MALA1, suggesting that prior events (such as nest initiation and Brood 1 development) must have occurred at roughly the same time. Nor could the delay at MALA1 be attributed to disturbances by olive grove farming because ANM regularly had local sheep and donkeys traversing the

aggregation. It was possible that the difference in the timing of Brood 2 was due to the difference in soil conditions (MALA1 may have had harder soils and therefore might have been a less suitable nesting site), difference in maturity of the two sites, or as a result of pesticide spraying at MALA1. The ANM aggregation was located in a garden that had been abandoned for more than 10 years and the soil conditions were rocky and clay-like, and nests were densely packed. The MALA1 aggregation was located on olive grove terraces that were formed 5 years ago (Richards pers. comm.), the soil was rocky and much harder than the soil at ANM, and the nests were spaced far apart. Irrigation pipes ran along the terraces at MALA1 that may have periodically flooded nests nearest to them. Moreover, at MALA1, frequent pesticide spraying may have slowed down the life cycle of most of the nests in the aggregation if nest occupants were killed while foraging (Richards pers. comm.).

Comparisons of the nesting phenology in Greece in 2000 with other European populations reveal that the third brood was produced earlier than the second brood in southern Spain and France (Knerer 1992). In Greece in 1998, the third brood was produced at about the same time as in 2000, but in 1998, consisted primarily of workers (with only one male and no gynes) (Richards 2000) (Table 1.7). The major difference among Greece and France and Spain was the length of the quiescent period during brood 2 development. In Greece, this lasted between 1 to 2 ½ weeks, but lasted 6 weeks in southern Spain and France. The variation in nesting phenology may be due to variation in local spring and early summer weather. During inclement weather, such as rain, foraging ceases while during cooler temperatures the developmental time slows down as in other halictines (Knerer 1992; Richards and Packer 1995, 1996).

Table 1.7: Nesting phenology of *L. malachurum* in southern populations.

Location/Month	April	May	June	July	August
S. Greece (Current study)	Brood 1 workers forage for Brood 2	Quiescent period (1-2 ½ weeks) Brood 2 workers emerge and forage for Brood 3	Quiescent period (<1week) Brood 3-workers, gynes and males develop Brood 3 occupants begin to emerge and Brood 3 workers forage	Brood 4?	
S. Greece (Richards 2000)	Brood 1 workers forage for Brood 2	Quiescent period (1-2 ½ weeks) Brood 2 workers emerge and forage for Brood 3	Quiescent period (1 week) Brood 3 workers emerge and forage	Aggregation closed early July (Quiescent period prior to Brood 4?)	
S. Spain and S. France (Knerer 1992)	Brood 1 workers emerge	Brood 1 workers forage for Brood 2	Quiescent period (6weeks)	Brood 2 workers emerge and forage for Brood 3	Brood 3 production begins

Colony demography

In Greece, 10 of 11 nests were founded by one queen, suggesting that most *L. malachurum* foundresses establish haplometrotic nests. In the other nest, however, two queens were found, suggesting that pleometrosis (multiple-founded nests) was not completely absent. The two queens were not very different in size, nor in their degree of ovarian development or degree of wear and tear, suggesting that both were actively involved in nesting activities such as cell construction, egg-laying and provisioning. In 1998, no queens were found in 6 nests excavated during June and so data regarding nest founding is unavailable for that year (Richards 2000). Knerer (1992) did not observe any multi-foundress nests in either northern or southern European populations of *L. malachurum*, suggesting that multi-foundress nesting was non-existent. *L. malachurum* gynes overwinter away from their natal nest (Knerer 1992) and so accidental encounters in spring among overwintered gynes from the same nest was unlikely. It is possible that on occasion the bi-phasic nest-founding phase results in two foundresses becoming tolerant of each other and co-inhabiting the same nest (Smith and Weller 1989).

The anomalous small queen that was found in nest ANM385 might have been a replacement queen that was originally a mated worker from the previous year who overwintered. This is not completely uncommon among the halictids. Richards and Packer (1998) suggested that undersized subordinate *H. ligatus* females in multiple-founded nests were workers from the previous year that had overwintered. In New York, *H. rubicundus* some first brood females overwinter and become foundresses the next year independent of body size (i.e. mated females are no larger than non-mated females) (Yanega 1989, 1992, 1993).

Foundresses provisioned the first brood of workers, which represented the only solitary phase of the colony life cycle. Means of 3.7 and 4.6 Brood 1 workers per nest were found at MALA1 and ANM respectively, based on nest excavations and observations

(Tables 1.4, 1.5). Richards (2000) observed a minimum of 0 to 5 workers per nest. Knerer (1992) reported means of 6.7 for England, 5.1 for Central France, 6.5 for southern Spain and 6.8 for southern France (Table 1.8). In Greece, Brood 1 consisted entirely of workers. In England 2.3% of the first brood was composed of males, in central France, 1.3%, in southern Spain up to 1.5% and in southern France up to 1.3% of the worker brood(s) consisted of males.

The size of Brood 2 was best represented from nests excavated during week 2, because in week 4 nests contained no provision masses or larvae, only pupae, indicating that brood production had ceased. Since some individuals could have reached maturity and left the nest by week 4, the number of brood found in nests excavated that week was an underestimate of the original number of brood laid. The average number of brood per nest, excluding parasites, in Brood 2 was 29 (Fig. 1.9), representing the mean number of workers produced in this brood since all brood 2 were workers. During this time, the number of adult workers foraging by the end of brood 2 (week 4) was 5 in one nest and 12 in another nest excavated, but each also included brood 1 workers that were still alive.

Richards (2000) found 14 and 16 adult workers in two fully excavated nests during this same period in the colony cycle. Knerer (1992) reported that in Austria, 15-30 worker pupae were found in nests excavated during mid-July (= Brood 2). He also stated that large colonies had several dozen foragers during the final provisioning phase, data that was possibly for Estepona, Spain (Knerer 1992).

Provisioning and cell construction of Brood 3 lasted 2 weeks, and was followed by an inactive period during week 6. Brood 3 started to emerge during week 7, marking the end of brood production, since only pupae and imagos were found in nests at this time. The mean number of Brood 3 offspring per nest was 48 and marked the commencement of the reproductive brood (gynes and males). The size of brood 3 reported in 1998 by Richards

Table 1.8: A comparison of behavioural and nesting characteristics of six European *Lasioglossum malachurum* populations.

Location	Population	No. worker broods	Mean no. 1st brood workers	Size of reproductive brood ⊕	Overall colony size	Proportion males in early brood(s) (%)	Proportion females mated (%)	Proportion females with developing ovaries (%)	Queen-worker size difference (overall) (%) (wing lengths)
North	**Isles of Wight, U.K.** †	1	6.7††	10-38	14-48	2.3	20	3.4††	14.3†† 15.4†
Central	**Dordogne, France**†	2	5.1††	56-249	71-249	1.3	8	30.1††	17.5†† 18.0†
South	**Spain**†	2	6.5††	52-112	85-167	0†-1.5††	0	12-61	18.0†† 19.0†
South	**Marseilles, France**†	3	6.8††	67-189	116-323	0-1.3	8	26	18.0†† 17.0†
South	**Greece***	3	4.0	-	mean=18.7	0	75	50-70	15.0++
South	**Greece****	3	4.6	mean=48.0	mean=85.6	0	0.7	58	12.0 11.0++

†Knerer (1992) ††Packer and Knerer (1985) *Richards (2000) **Current study

++ Based on head width measurements

⊕ Size of reproductive brood: Brood 2 in England, Brood 3 in Spain and central France, Brood 4 in southern France. No reproductive brood was reported in Greece in 1998.

(2000) was substantially smaller than in 2000, with a mean brood size of approximately 4 (based on four fully excavated nests and including filled in brood cells!). Knerer found up to 34 workers (based on 9 nests) in the Tulln, Austria population, and up to 53 Brood 3 workers (numbers based on several nests, actual number of nests not provided) in the Les Eyzies, France population (Knerer 1992). The size of the reproductive brood produced in other populations (compared to Brood 3 in Greece) ranged from 10-38 individuals in England, 56-249 in central France, 52-112 in southern Spain, and 67-189 in southern France (Knerer 1992) (Table 1.8).

Based on head width measurements and size comparisons of Brood 3, 16 (18%) females were workers. Brood 3 production ended in late June and it seems unlikely that this represents the end of the colony cycle. I suggest that the colony cycle of the Greek population was not complete and that these workers would have provisioned a fourth brood. Knerer (1992) reported that a third worker brood, followed by a fourth brood of reproductives, existed in most of the Mediterranean region and he reported the presence of males and gynes in August and September in Mediterranean populations (Knerer 1992). Richards (2000) caught worker-sized females on July 19th, 1998, providing further evidence that in Greece, in 2000, a fourth brood was yet to be produced.

The numerical sex ratio can provide information regarding investment ratio skews. For example, a biased numerical sex ratio in favor of females indicates workers gain more, in terms of inclusive fitness, from raising sisters rather than brothers because of a higher degree of sister-sister relatedness, thus providing an explanation for low worker reproduction (and therefore, a high degree of worker altruism) (Trivers and Hare 1976). The overall Brood 3 numerical sex ratio of females (workers and gynes) to males was 1.2:1 and not significantly different from 1:1. The observed sex ratio of gynes to males was 1:1, however sex and caste assignment for 43% of Brood 3 was not possible and so, by not including these individuals, a precise sex ratio was not realized. Knerer (1992) found

female biased numerical sex ratios in smaller nests in southern populations. More populous nests, however, yielded male biased sex ratios (Knerer 1992). He attributed this to the limited number of eggs a queen can lay per day compared with the number of foraging trips performed by workers per day. Queens can lay a maximum of 6 eggs per day (Knerer 1992), a number that can be provisioned by a small number of workers. Nests with many workers can provision up to 20 cells per day, so the extra provisions provide an opportunity for workers to lay their own male-destined eggs, thereby increasing the number of males to females (Knerer 1992).

The investment ratio provides information regarding control over resource allocation to future offspring, i.e. whether the queen or the workers control the investment ratio (Trivers and Hare 1976). The investment sex ratio is defined as the relative amount of resources, such as the amount of food, allocated to offspring of one sex compared to the other. This can be estimated by determining the dry-weights at emergence of both sexes, based on the assumption that the relative cost to the investor of an offspring of a given sex is proportional to the relative weight of an average individual of that sex (Danforth 1990). Investment ratios (gynes to males) based on dry-weight measurements ranged from 0.3:1 to 2.3:1 in individual nests, with an overall ratio of 1.4:1. Like the sex ratios, this provided only an estimate of Brood 3 since 43% of the brood were still larvae and so sex or caste could not always be determined. An investment ratio favouring gynes would suggest that workers control the investment ratio rather than queens, which would prefer a 1:1 ratio of equal investment (Trivers and Hare 1976). In Ontario, *Halictus ligatus* workers gain control over the sex ratio and skew resource allocation in favour of their sisters and daughters and away from their brothers because they are able to evade the control of the aging queen (Richards et al., 1995).

The mean age of Brood 3 males was statistically higher than that of females (workers and gynes), indicating that Brood 3 was protandrous. Since males emerged before gynes, the

observed sex ratio of 1:1 may be a result of gynes still being in the larval stage and therefore not counted, lending support to sex and investment ratios in favour of gynes.

Protandry may have important implications for mating behaviour in *L. malachurum*. Males that emerge prior to gynes may have more success at finding a receptive female than males that emerge at the same time or after them (Crozier and Pamilo 1996). From the queen's point of view, therefore, males are more valuable to her when they are produced and emerge before gynes since males that emerge after gynes may lose mating opportunities. If this is true, the order in which Brood 3 gynes, workers and males emerge may influence mating opportunities available to Brood 3 workers. The age distribution of Brood 3 offspring (Fig. 1.11) showed a modest difference between the age of gynes versus workers, the latter having more individuals that were in older age categories. Although sample sizes were small, Brood 3 workers emerging a few days before gynes would have had an opportunity to mate with the males that had already emerged, potentially changing the social dynamics within the nest. This may have been the case in 1998 because Richards (2000) found that 75% of mid-summer workers were mated with well-developed ovaries and were therefore capable of laying both male and gyne eggs. She suggested that gynes and males produced in the final brood in many nests could have been the offspring of these fertile workers, and that *L. malachurum* colonies switch from eusocial to semisocial organization. In 2000, the production of workers and males in Brood 3, along with an increase in the incidence of mated workers in week 7, suggested that if Brood 4 was still to be produced, some of them would have been the offspring of Brood 3 workers.

The timing of male production had an effect on the proportion of reproductive workers in *H. ligatus* in Ontario in different years (Richards and Packer 1995). Males that were produced late in the first brood in 1984, along with protandry in the second brood, meant that males would have mated mainly with gynes. In 1991, however, an overlap of brood 1 (the worker brood) and brood 2 (the reproductive brood) production and early

emergence of males in the first brood created a scenario that may have increased the opportunity for males to mate with both workers and gynes (Richards and Packer 1995). Similarly, the timing of male production relative to worker emergence is an important factor influencing the strength of eusociality in *H. rubicundus*. In New York, *H. rubicundus* castes are determined by whether a female mates or not (females that mate become gynes and overwinter, females that do not mate in the first 2 days post-emergence become workers), therefore, males must be rare relative to virgin females if any of them are to remain unmated long enough to become workers (Yanega 1993).

Parasites

The presence of a guard at the nest entrance suggests that guarding is an adaptation to strong parasite pressure (Brothers et al., 2000). Parasitic bombyliid larvae were found in brood cells during all weeks except week 7. The highest incidence of parasitism occurred at the end of Brood 2, and the beginning of Brood 3, suggesting that guarding was not completely effective during weeks 4 and 5. Although the two nests excavated during week 7 were only partially dug, no parasites were found in brood cells. A reduction in the proportion of parasites found later in the colony cycle may be as result of bombyliid parasites no longer present during June, or if they were still present, an increased depth of Brood 3. This brood was found from 55 – 94 cm below the nest entrance and the top part of the tunnel was blocked with soil. Furthermore, the presence of a guard in open nests and a greater number of workers probably improved nest defense in comparison to earlier in the colony cycle when there were fewer workers to help defend the nest.

Characteristics of queens and workers

Size dimorphism between queens and workers

The degree of size dimorphism between queens and their daughter workers has been suggested to influence the strength of colony eusociality (Breed 1976; Packer and Knerer 1985). Queen control of worker behaviour is through physical domination, which is more effective when queens are substantially larger than their daughters (Michener 1974; Kukuk and May 1991; Crozier & Pamilo 1996; Smith & Weller, 1989). Queens were 10.6% larger than workers, with a range of 5.4% to 14% in individual nests (6.5% to 17.9% based on wing length measurements). These size differentials are relatively large for halictine sweat bees (Breed 1976; Packer and Knerer 1985) and agree with Knerer's (1992) and Stöckhert's (1923) descriptions of *L. malachurum* as having a well established caste system. In Greece, Richards (2000) reported size differences between *L. malachurum* queens and workers of 12-16%. Knerer (1992) reported mean size differences between queens and workers of 15.4% in the Isle of Wight, England, 18% in central France, 19% in southern Spain, and 17% in southern France (Table 1.8), based on wing lengths and abdomen widths. Comparisons of abdominal width may be problematic if females exhibited different degrees of ovarian development, or there were different amounts of food in the gut or pollen sac or, in the case of imagos, their abdomens were full of fat stores, as was observed in this study. Packer and Knerer (1985) reported slightly different numbers, 14.3% in England, 17.5% in central France (Dordogne), 18% in Estepona, Spain and Marseilles, France. Knerer (1992) did not report size dimorphism ranges, but in Greece, the maximum size difference based on wing lengths was 18%, which is similar to other populations and suggests strong caste differentiation in this species across its geographic range.

Reproductive skew

In Greece, queens were the primary reproductives and workers were primarily altruistic. There are five lines of evidence for this. (1) Upon emergence of the first brood of workers, the queen ceased foraging activity and presumably remained in the nest, while her workers actively foraged and provisioned subsequent broods. (2) Most workers (58%) had high mandibular wear scores that increased over time, suggesting a high contribution to nesting activities. (3) All the queens were mated (except for one, for which matedness could not be determined). (4) Overall, the highest ovarian development score for queens was 5 (n=14), whereas the highest score observed in workers was 2.75 (n=297). (5) In excavated nests, queens had much higher total ovarian development scores than all the adult workers in the same nest. Furthermore, it seems apparent that *L. malachurum* workers were not laying gyne-destined eggs in Brood 3 because only 0.7% of workers were mated.

Suppression of worker egg-laying by the presence of relatively large queens was possible for most Brood 1 and 2 workers, however, a few workers may have laid male eggs. Twenty-eight percent of Brood 1 and 2 workers had fully mature eggs ready to lay that were potentially destined to be males in Brood 3. If workers were involved in male production, then I expected to find an increase in worker ovarian development prior to, and at the onset of, male production. At the end of male production worker ovarian development should have declined. This trend was observed for *L. malachurum* in southern Greece. Of 84 workers with at least one ½-developed oocyte, 29% and 43% (weeks 4 and 5 respectively) of workers had ovarian development suggestive of egg-laying one week prior to and at the onset of male production. These numbers showed a decrease near the end of male production. Furthermore, the highest worker ovarian development score (2.75; Fig. 1.16) was observed in a worker that was caught leaving a nest during week 4 (nest ANM339, W3). In Tulln, Austria, *L. malachurum* workers were apparently involved in male egg

laying and there was an increase then decrease in worker ovarian development that correlated with the timing of male production (Knerer 1992). Across *L. malachurum*'s range, populations showed an increasing proportion of workers with developed ovaries as populations were found in more southern regions, suggesting a cline of increasing worker reproduction (Knerer 1992; Richards 2000; this study).

Caste distinction in an obligately eusocial sweat bee

Richards (2000) discussed the problem of caste-associated behaviour in socially labile sweat bees; Caste-specific roles are associated with the degree of worker altruism and the strength of eusociality. In Greece, it was clear that there is no confusion as to caste-specific roles in *L. malachurum*. Workers were very helpful since they performed all foraging activity for Broods 2 and 3, and were not the primary reproductives. Furthermore, workers were significantly smaller than queens, with virtually no size overlap, suggesting that queens may have been able to manipulate worker behaviour (Kukuk and May 1991). Previous observations (Knerer 1992) suggested that workers' abdomens do not have fat bodies, whereas gynes have abdomens full of fat bodies that allow them to survive overwintering diapause and to become foundresses the following spring, as seen in *H. ligatus* (Richards and Packer 1994). Knerer suggested that in *L. malachurum*, the fat bodies induce diapause regardless of the size of the female. In the southern Greek population, however, my dissections of gyne, worker and male imagos indicated that all newly emerged *L. malachurum* had abdomens full of fat bodies. Furthermore, 2 of 16 females with abdominal fat bodies had worn mandibles suggesting that these individuals were not newly eclosed and had performed some level of activity. Richards (2000) also found some workers in 1998 with abdominal fat bodies. Consequently, caste differentiation based on this criterion is not reliable for this species.

Evidence for temporal and geographical variation

In Greece, the differences between 1998 and 2000 in a number of nesting behaviours were striking. In 1998, compared to 2000, colony sizes were substantially smaller, queens were absent by Brood 3, and there was an extremely high proportion of reproductively active workers (Richards 2000). A possible explanation for the huge annual variation was pesticide spraying. The aggregation studied in 1998 was located in olive grove terraces where frequent pesticide spraying was so intense that observations and excavations had to cease (M.H. Richards, pers. comm.). If spraying poisoned most foragers then this could account for aberrantly small brood sizes in 1998. Moreover, the absence of the queens would account for increased worker reproduction in order to ensure the production of the reproductive brood. The overall result would be a switch in colony social organization from eusocial to semisocial (Richards 2000).

Comparing the social and nesting characteristics of *L. malachurum* in Greece with other European populations (Table 1.8), the hypothesis of a north-south cline of decreasing eusociality is supported. The most noteworthy variation was observed in the length of the colony cycle, colony size and the proportion of workers with developed ovaries. It is predicted that highly eusocial insects have larger colonies, as is the case for many ant species and honey bees (Michener 1974; Crozier and Pamilo 1996), however, in the case of *L. malachurum* in Greece, weaker eusociality appears to be associated with larger colony sizes. The unvarying characteristics included queen monopolization of female oviposition (because very few workers were mated). With the exception of the Greek 1998 population, in no populations were mated workers with developed ovaries reported (Packer and Knerer 1985; Knerer 1992; Richards 2000). Other unvarying characteristics included no substantial variation in caste size dimorphism; in all populations a large size difference with almost no overlap was observed. Finally, in all populations most nest founding was haplometrotic (singly founded) and most nests had a guard present at the nest entrance. Therefore, the

variable traits must define social level in *L. malachurum* across its geographic range. It was of interest to test whether the variable traits that vary within *L. malachurum*, and therefore describe intraspecific social variation, are also the traits that vary among species and can be used to define social variation within *Evylaeus*.

An explanation for social variation in L. malachurum

For this obligately eusocial species it appears that the length of the breeding season most appreciably affects colony social organization. A gradient of increasing lengths of summer seasons from north to south coincide with an increase in the number of worker broods and larger colony sizes. Larger colonies mean that workers can provision more eggs than the queen may be able to lay, providing opportunities for workers to lay their own eggs. In England, where only one worker brood is produced, only 3.4% of workers had developed ovaries. Furthermore, queen-worker size dimorphism was similar to that reported for other populations suggesting that queen control over a comparatively small number of workers was plausible. More southerly populations have longer summers that may increase the probability of early queen death, before the production of the reproductives. If this happens, workers must take over the job of laying the gyne and male eggs and the strength of colony eusociality weakens, or even switches to semisocial, as appeared to be the case in Greece in 1998 (Richards 2000).

Conclusions

Discerning the evolution and maintenance of altruism and eusociality requires that we understand the factors that lead to intraspecific as well as interspecific social variation. Obtaining this understanding requires an in-depth knowledge of the life cycle and social biology of socially variable species.

Lasioglossum malachurum is an obligately eusocial species that exhibits intraspecific geographic variation. Knerer (1992) suggested that *L. malachurum* represents the paradigm of a strongly eusocial sweat bee because of a well established caste system of reproducing queens and altruistic workers. My findings support this paradigm. In Greece, two worker broods were produced, and these non-reproductive workers actively foraged and constructed cells, as indicated by high degrees of mandibular wear. Males were not produced until Brood 3. Queens were the primary reproducers as indicated by the majority of workers without well developed ovaries early in the colony cycle. There was a large size difference between most queens and workers, and queens survived well into the third brood. All of these characteristics are indicative of a well established caste system and a high degree of worker altruism.

Direct reproduction of male offspring decreases the degree of altruism by *L. malachurum* workers, decreases the strength of eusociality and lends support to the hypothesis of a cline of decreasing eusociality. I report potential worker contributions to male offspring because many workers showed some degree of ovarian development later in the colony cycle and 28% of them had ovarian development that coincided with male production, thereby decreasing the strength of eusociality during Brood 3 production. The strength of eusociality in *L. malachurum* appears to be under environmental influence because as the length of the summer season increases, colonies become larger and workers potentially gain reproductive advantage over the queen (Dunn et al., 1998; Wcislo, in Choe and Crespi, 1997).

If environmental factors such as the length of the season influence colony demography and therefore the social status of different populations of *L. malachurum*, will these be the same factors that will tend to associate with social variability in *Evylaeus* as a whole? In Chapter Two, a comparative study using principal components analysis is presented that will address this question and determine the characteristics that tend to

associate with social variation among solitary, eusocial and socially polymorphic *Evylaeus* species and populations.

CHAPTER TWO

A comparative analysis of social variation in *Evylaeus*

One of the main goals of studying the evolution of social behaviour is to determine the behavioural characteristics that are required for selection to favour one social system over another (e.g. solitary versus eusocial, or weak versus strong eusociality). These characteristics may promote or discourage worker altruism. The subgenus *Evylaeus* is an excellent group for studies of social evolution because it contains numerous species that express solitary, eusocial or socially polymorphic behaviour. Based on phylogenetic studies, eusociality appears to be the ancestral state and at least four independent losses of eusocial behaviour have been proposed (Danforth 1999; Packer 1991). Understanding the evolution and maintenance of altruism and eusociality require that we understand the factors that lead to both interspecific and intraspecific social variation. Obtaining this understanding requires an in depth knowledge of the behavioural life cycle and social biology of socially variable species.

It was concluded in Chapter One that in Greece, *L. malachurum* is strongly eusocial because of a well established caste system of queens that monopolized reproduction and workers that were primarily altruistic. It was also concluded that my results substantiate intraspecific social variation and support the hypothesis of a north-south cline of decreasing eusociality, because of an increased proportion of workers potentially involved in male production in Greece compared to more northerly populations. Other differences in Greece compared to other European populations were a higher number of worker broods and an overall increase in colony size. These differences were attributed to variation in the length of the summer season that would promote the production of larger colonies and provide opportunities for workers to evade queen control in Greece.

The objective of Chapter Two is to determine whether patterns of social variation within *L. malachurum* reflect patterns within *Evylaeus* as a whole. Are the variable traits that appear to influence colony demography in *L. malachurum* the same ones that tend to associate with varying levels of eusociality in other species? If not, how different is *L. malachurum* from the rest of the subgenus? A comparative study was performed, using principal components analysis, on solitary, eusocial and socially polymorphic *Evylaeus* species and populations in order to uncover the characteristics that tend to associate with social variation within this subgenus.

Principal components analysis explores the correlational patterns within large data set and seeks to extract the 'principal components' of this variation. A principal components analysis creates a multidimensional picture of the relationships among the variables, by reducing the original data set into smaller sets of linear combinations that explain as much of the original variance as possible. Because the analysis involves multiple dimensions or components, and each component accounts for a different amount of the variation, it is the principal components that account for the most variance that prove most useful. Based on the correlations of the variables on each principal component, inferences can be made about the biological significance of a component.

Previous comparative studies using principal components analysis

Previous studies by Michener (1974), Breed (1976) and Packer and Knerer (1985) used principal components analyses to analyze behavioural characteristics in attempts to determine which behavioural characteristics are most closely related to social evolution. Michener (1974) analyzed twenty-eight behavioural and nesting characteristics from eighteen species in the subfamilies Halictinae (tribes Halictini and Augochlorini) and Apinae (tribes Bombini and Allodapini). Michener found that the behavioural and social traits that he used distinguished among taxonomic groupings, rather than social type,

indicating that the course of social evolution is likely different in different families (Michener 1974; Packer and Knerer 1985). The principal components analysis did reveal, however, that there was a distinct distribution of species associated with social level, with solitary and primitively eusocial species clustered closer together and away from the highly eusocial species. Michener concluded that biological attributes used to describe solitary and primitively eusocial species were similar to one another and therefore evolutionary changes from solitary to primitively eusociality would be relatively easy. Compared to the highly eusocial Apinae, for which a major evolutionary change would require changes in a variety of characteristics and would take a significant amount of time (Michener 1974).

Breed (1976) provided a similar analysis of eusocial behaviour for primitively eusocial bees in the subgenus *Lasioglossum* (*Dialictus*). Breed's objectives were to examine a number of behavioural traits of social importance in order to determine which characteristics were most closely related to social evolution, and to determine an inter-species social ranking from weak to strong. Breed's principal components analysis included six obligately eusocial *Dialictus* that showed a range of social behaviours. Five behavioural traits were considered to be most closely associated with the expression of eusociality: (1) the percent size difference between queens and workers, (2) the number of bees per nest, (3) the percent seasonal size variation in workers, (4) queen longevity, and (5) the number of queens per nest.

Similarly, Packer and Knerer (1985) analyzed eleven behavioural characteristics of eight species of the subgenus *Lasioglossum (Evylaeus)* and found that six traits were highly correlated with stronger eusociality, including a reduction in the proportion of males in the first brood, a reduction in the proportion of workers that mate, a reduction in the proportion of workers that have developed ovaries, an increase in the mean number of workers in the first brood, an increase in the size difference between queens and workers, and the construction of cavities built around the brood area within nests. Based on the correlated

expression of these highly significant traits with respect to the first component, a ranking of relative social level showed that *L. laticeps* was the most weakly eusocial and *L. malachurum* and *L. marginatum* were the most strongly eusocial *Evylaeus* species.

In this chapter, I expand Packer and Knerer's (1985) analysis of social variation in *Evylaeus* to include species and populations whose social behaviour has been studied since 1985. Most critically, I include in my analysis, several populations of solitary and socially polymorphic bees, unavailable to Packer and Knerer.

Materials and methods

Data for behavioural and nesting characteristics for *Evylaeus* and *Lasioglossum* species, populations and study years were available from Packer and Knerer (1985) and more recent field studies (Tables 2.1 and 2.2). The principal components analysis presented in this study included three solitary species that were absent from Packer and Knerer's (1985) study on *Evylaeus*. Furthermore, additional *L. malachurum* populations and socially polymorphic *Evylaeus* species were added to the analysis. Two *L. calceatum* populations, two *L. apristum* populations (solitary and eusocial) and the *L. cooleyi* population were excluded from the analysis due to missing data. The principal component analyses were programmed using SAS® and graphing was performed using SigmaPlot 2000®.

Nine nesting and social traits were used in the analysis. These traits were: (1) The type of nest founding by queens in the spring. Nest founding may be haplometrotic, when an overwintering female founds a nest alone in the spring, or pleometrotic, when two or more overwintered females inhabit the same nest at the beginning of the season. (2) The mean number of first brood workers (solitary populations do not produce any workers, so values of 0.0 were used). (3) The proportion of females with developed ovaries. (4) The proportion of females mated. Females of social populations included the proportion of population wide workers with developed ovaries only, and for solitary populations included

Table 2.1: A quantitative comparison of behavioural characteristics for different *Lasioglossum (Evylaeus)* species, populations and study years from varying localities.

Species	Locality	Type of nest founding 0=haplo 1=pleo	Mean no. 1st brood workers	% females with developed ovaries	% females mated	% Size difference (Q>W)	% males in 1st brood(s)	Gynes overwintering location 0=in nest 1=away from nest	No. Worker broods	Guard? 0=no 1=yes
Evylaeus albipes	**Dordogne, France** (Plateaux-Quenu 1992)	1	3.0 (1 lab reared nest)	0.0	100 (lab reared nests)	5.6**	20-50 mean=35.0⊗ (lab reared nests)	0	1	0
Evylaeus apristum	**Japan** (Miyanaga et al.,1999)	0	6.9 (green-house raised)	39 (4 nests) 8.7 (flower caught ♀'s)	0.0 (4 nests) 4.4 (flower caught ♀'s)	7.7**	5.5 (green-house raised)	1 (13/16 dug new burrows)	1	-
Solitary *Evylaeus apristum*	**Northern Japan** (Miyanaga et al.,1999)	0	0.0	100	100	0.0	-	-	0	0
Evylaeus baleicum	**Nishioka Japan** (Cronin and Hirata, unpub.)	0 (0.9% pleo)	2.8	26	50	6	12-16 mean=14.0⊗	1	1	1

⊗ data used in PCA * Calculations based on wing length ** Calculations based on head width

Table 2.1 continued…

Species	Locality	Type of nest founding 0=haplo 1=pleo	Mean no. 1st brood workers	% workers with developed ovaries	% workers mated	% Size difference (Q>W)	% males in 1st brood(s)	Gynes overwintering location 0=in nest 1=away from nest	No. Worker broods	Guard? 0=no 1=yes
Solitary ***Evylaeus baleicum***	**Kawakita Japan** (Cronin and Hirata, unpub.)	0	0.0	100	100	0.0	50.0	1	0	0
Evylaeus calceatum	**Okusawa, Japan** (Sakagami and Munakata 1972, in Packer & Knerer 1985)	1 (11% pleo)	-	18.6	16.3	5.0*	-	0	1	1
Evylaeus calceatum	**Dordogne, France** (Plateaux-Quenu 1992)	1	12 (max.)	-	85.0	16.0**	18.0 (lab reared nests)	0	1	0
Evylaeus cooleyi	**Vancouver, British Columbia** (Packer & Owen 1989)	0	1.3	39.7	14.5	7.3* 5.1**	-	-	1	-
Evylaeus duplex	**Hokkaido, Japan** (Sakagami and Fukuda 1975, 1989, in Packer and (Knerer 1985)	0 (0.8% pleo)	4.6	20.2	8.5	9.6	10.0	0	1	1

⊗ data used in PCA * Calculations based on wing length ** Calculations based on head width

Table 2.1 continued...

Species	Locality	Type of nest founding 0=haplo 1=pleo	Mean no. 1st brood workers	% females with developed ovaries	% females mated	% Size difference (Q>W)	% males in 1st brood(s)	Gynes overwintering location 0=in nest 1=away from nest	No. Worker broods	Guard? 0=no 1=yes
Evylaeus laticeps	**Dorset, England** (Packer 1983[+]; Packer & Knerer 1985)	0	4.1	53.0	18.0	7.3[*⊗] 4.5[**+]	23.8	1	1	1
Evylaeus lineare	**Paris, France** (Knerer 1983, in Packer & Knerer 1985)	1	4.1	13.6	0.5	15.0[*]	1.0	0	1	1
Evylaeus lineare	**Dordogne, France** (Knerer 1983, in Packer & Knerer 1985)	0	6.3	3.2	0.5	21.0[*]	1.5	0	1	0
Evylaeus malachurum	**IOW, England** (Knerer 1992[+]; Packer and Knerer 1985[++])	0[+]	2.3[++]	3.4[++]	4-20.0[+] (flower caught ♀'s) mean=12.0[⊗]	15[*]	2.3[+]	1	1	1
Evylaeus malachurum	**Dordogne, France** (Knerer 1992[+]; Packer and Knerer 1985[++])	0[+]	2.0[++]	8[+⊗] 30.1[++]	8.0[+] 0.0[++]	W1: 17.5[*] W2: 13.6[*] mean=15.8[*⊗]	2.0[+]	1	2	1

⊗ data used in PCA *Calculations based on wing length **Calculations based on head width

Table 2.1 continued...

Species	Locality	Type of nest founding 0=haplo 1=pleo	Mean no. 1st brood workers	% females with developed ovaries	% females mated	% Size difference (Q>W)	% males in 1st brood(s)	Gynes overwintering location 0=in nest 1=away from nest	No. Worker broods	Guard? 0=no 1=yes
Evylaeus malachurum	**Estepona, Spain** (Knerer 1992[+]; Packer and Knerer 1985[++])	0[+]	1.5[++]	12-61[+] (mean=36.5)[⊗] 17.6[++]	0.0[+]	W1: 18.1[*] W2: 14.1[*] mean=16.6[*⊗]	0[+] 1.5[++⊗]	1	2	1
Evylaeus malachurum	**Marseilles, France** (Knerer 1992[+]; Packer and Knerer 1985[++])	0[+]	6.8[++]	16.3-34.6 mean=25.5[+⊗]	5.8[+⊗] (3 nests) 0.0[++]	W1: 17.0[*] W2: 17.0[*] W3: 13.8[*] mean=15.9[*⊗]	0[+]-1.3[+⊗]	1	3	1
Evylaeus malachurum	**Agios Nikolaos, Greece 1998** (Richards 2000)	0	4.0	67.0 (flower caught ♀'s)	50.0-70.0 (flower caught ♀'s) mean=60[⊗]	14.3[**] (1 queen and 35 workers from nests)	-	1	3	1
Evylaeus malachurum	**Agios Nikolaos, Greece 2000** (This study)	0 (8.3% pleo)	4.0	58.0	0.7	W1: 8.7[**] W2: 11.0[**] W3: 8.4[**] Overall: 10.6[**⊗]	0.0	1	3	1

W1 = size difference between queens and first brood workers. W2 = size difference between queens and second brood workers. W3 = size difference between queens third brood workers.

⊗ data used in PCA * Calculations based on wing length ** Calculations based on head width

Table 2.1 continued...

Species	Locality	Type of nest founding 0=haplo 1=pleo	Mean no. 1st brood workers	% females with developed ovaries	% females mated	% Size difference (Q>W)	% males in 1st brood(s)	Gynes overwintering location 0=in nest 1=away from nest	No. Worker broods	Guard? 0=no 1=yes
Evylaeus marginatum	**Dordogne, France** (Plateaux-Quenu 1959, in Packer & Knerer 1985)	0	3.5	0.0	0.0	0.1*	0.0	0	5	0
Evylaeus marginatum	**Greece** (MH Richards unpub. data)	0	3.5	0.0	0.0	0.1*	0.0	0	5	1
Evylaeus nigripes	**Dordogne, France** (Knerer & Plateaux-Quenu 1970, in Packer & Knerer 1985)	1	7.2	59.0	1.0	10.3**	4.0	0	1	0
Evylaeus pauxillum	**Tulln, Austria** (in Packer & Knerer 1985)	0	4.0	15.0	1.0	14.5*	5.0	1	2	1
Solitary *Evylaeus villosulus*	**Paris, France** (Plateaux-Quenu et al., 1989)	0 (5% pleo)	0.0	100	100	0.0	17.0	1	0	0

⊗ data used in PCA *Calculations based on wing length **Calculations based on head width

Table 2.2: A quantitative comparison of behavioural characteristics for two *Lasioglossum (Lasioglossum)* species.

Species	Locality	Type of nest founding 0=haplo 1=pleo	Mean no. 1st brood workers	% females with developed ovaries	% females mated	% Size difference (Q>W)	% males in 1st brood(s)	Gynes overwintering location 0=in nest 1=away from nest	No. Worker broods	Guard? 0=no 1=yes
Solitary *Lasioglossum scitulum*	**Greenhouse, Japan** (Miyanaga et al., 2000)	1	0.0	100	100	0.0	46.0	1	0	0
Solitary *Lasioglossum mutilum*	**Greenhouse, Japan** (Miyanaga et al., 1998)	1 (15% pleo)	0.0	100	100	0.0	53.6	0	0	0

*Calculations based on Wing length **Calculations based on head width

foundresses (no workers are produced in solitary populations). (5) The population mean percent size difference between queens and workers. This was calculated as:

$$\frac{\text{mean queen head width} - \text{mean worker head width}}{\text{mean queen head width}} \times 100$$

(6) The proportion of males in the first brood(s). Data for social species or populations were applicable for worker broods only, not including the reproductive brood. In solitary species, the first brood always consists of both males and females. (7) The overwintering location of gynes. Gynes may either dig overwintering burrows within their natal nest or away from their natal nest. (8) The number of worker broods. (9) Nest guarding. Traits (1), (7) and (9) were qualitative data and required coding before being used in the analysis. Data for all the other traits were quantitative and so numbers could be used directly in the analysis.

Data regarding the type of nest founding (1) were coded as either 0 for haplometrosis (single-female nest founding) or 1 for pleometrosis (multifemale nest founding). The overwintering locale of gynes (7) was coded as 0 for species in which gynes overwinter within the natal nest and 1 for species in which gynes overwinter away from the natal nest. A coding of 1 for this trait was assigned if more than 10% of nests in a population were polygynous since this number is comparatively high for *Evylaeus*. Nest guarding (9) was coded as 1 for nests guarded during spring and/or summer or 0 for nests unguarded, following Packer and Knerer's (1985) coding. For solitary species, the proportion of mated females and the proportion of females with developed ovaries were both reported as 100% because it would be expected that all solitary females are foundresses, therefore, 100% should be mated and have developed ovaries.

A second principal components analysis was performed that excluded the solitary populations in order to compare the differences in social level among the eusocial

populations. The *L. marginatum* populations were also excluded because this species is so different from the other eusocial *Evylaeus*.

Interpreting the results of the principal components analysis

In a principal components analysis (PCA), the number of principal components is equal to the number of original variables used. In this case, nine variables (= behavioural characteristics) were used. The goal of PCA is to account for as much of the total variance with as few principal components as possible (Dillon and Goldstein 1984). The first principal component accounts for the largest portion of the total variance in the data, the second principal component accounts for the second largest amount of the remaining total variation, and so on. Each successive principal component is uncorrelated with the preceding principal component (Dillon and Goldstein 1984; Krzanowski 1988).

In order to determine the number of principal components to retain in the analysis, two criteria were applied. The first was to choose those principal components that had eigenvalues greater than one, referred to as the "root greater than one" criterion (Dillon and Goldstein 1984). The second was to choose the first 'x' eigenvalues that explained greater than or equal to 80% of the total variance, which was sufficient to capture the variance structure of the original data set. Principal components contain the component weights or loading factors of the variables. The loading factors indicate the strength of the correlations among the variables and the degree to which each variable contributes to defining the corresponding principal component. A variable was considered significant for defining a particular principal component if the loading factor was greater than 0.70 (Dillon and Goldstein 1984). Loading scores greater than 0.60 were considered moderately significant to defining a component.

Results

Data for seven primitively eusocial *L. (Evylaeus)* species, two socially polymorphic species (*L. albipes* and *L. baleicum*), and three solitary species (*L. (E.) villosulus*), *L. (Lasioglossum) mutilum* and *L. (L.) scitulum*) were used in the analysis. Of the seven eusocial species, six *L. malachurum*, two *L. lineare*, and two *L. marginatum* populations were included, along with one population of *L. duplex*, *L. nigripes*, *L. laticeps* and *L. pauxillum*. In total, 20 species and populations were included in the analysis.

Table 2.3 shows that 84% of the total variance is explained by the first three principal components and the eigenvalues drop below one after three components. It is these components, therefore, that are retained for analyses.

Six characteristics have loading scores ≥0.70 on principal component one, which explains 52.4% of the total variance. These are, in order of factor loading and associated with stronger eusociality, a decrease in the proportion of males in the early brood, a decrease in the proportion of mated females, and increase in the mean number of first brood workers, a decrease in the proportion of females with developed ovaries, a larger percent size differential between queens and workers and the presence of a guard at the nest entrance.

Principal components 2 and 3 explain 19% and 13% of the original variance, respectively. Overwintering locale of gynes is the only trait with a significant loading score on component two (0.95) and the number of worker broods has the highest loading score on component three (-0.70).

Three-dimensional plots of the first three principal components along with the behavioural and social characteristics that loaded heaviest on these components, (Figures 2.1 and 2.2), shows a clear distinction can be observed between the eusocial and the solitary species, with the socially polymorphic *L. albipes*, falling in between (Figure 2.1). The separation of the eusocial populations from the solitary populations is driven by principal

Table 2.3: Results of the principal components analysis for *Evylaeus*. Loading factors scores of ≥0.70 were considered significant, and ≥0.60-0.69 were considered moderately significant, to defining a principal component.

	Loading factors		
Variable	**Factor 1**	**Factor 2**	**Factor 3**
Type of nest founding	0.53362	-0.58874	0.40314
% Males in early brood	0.92563	0.00530	0.09026
% Females mated	0.91790	0.16763	-0.00813
% Size difference Q>W	-0.73374	0.08706	0.60781
Overwintering locale	-0.02769	0.95058	0.08356
% Females with developed ovaries	0.76086	0.39876	0.07185
No. of worker broods	-0.60512	-0.17389	-0.70029
Mean no. of 1st brood workers	-0.86634	-0.15299	0.35264
Presence or absence of a guard	-0.70532	0.41906	0.02247
Eigenvalue	**4.7163**	**1.6742**	**1.1676**
Percent Explained	**52.4**	**18.6**	**12.9**
Cumulative Percent	**52.4**	**71.0**	**83.9**

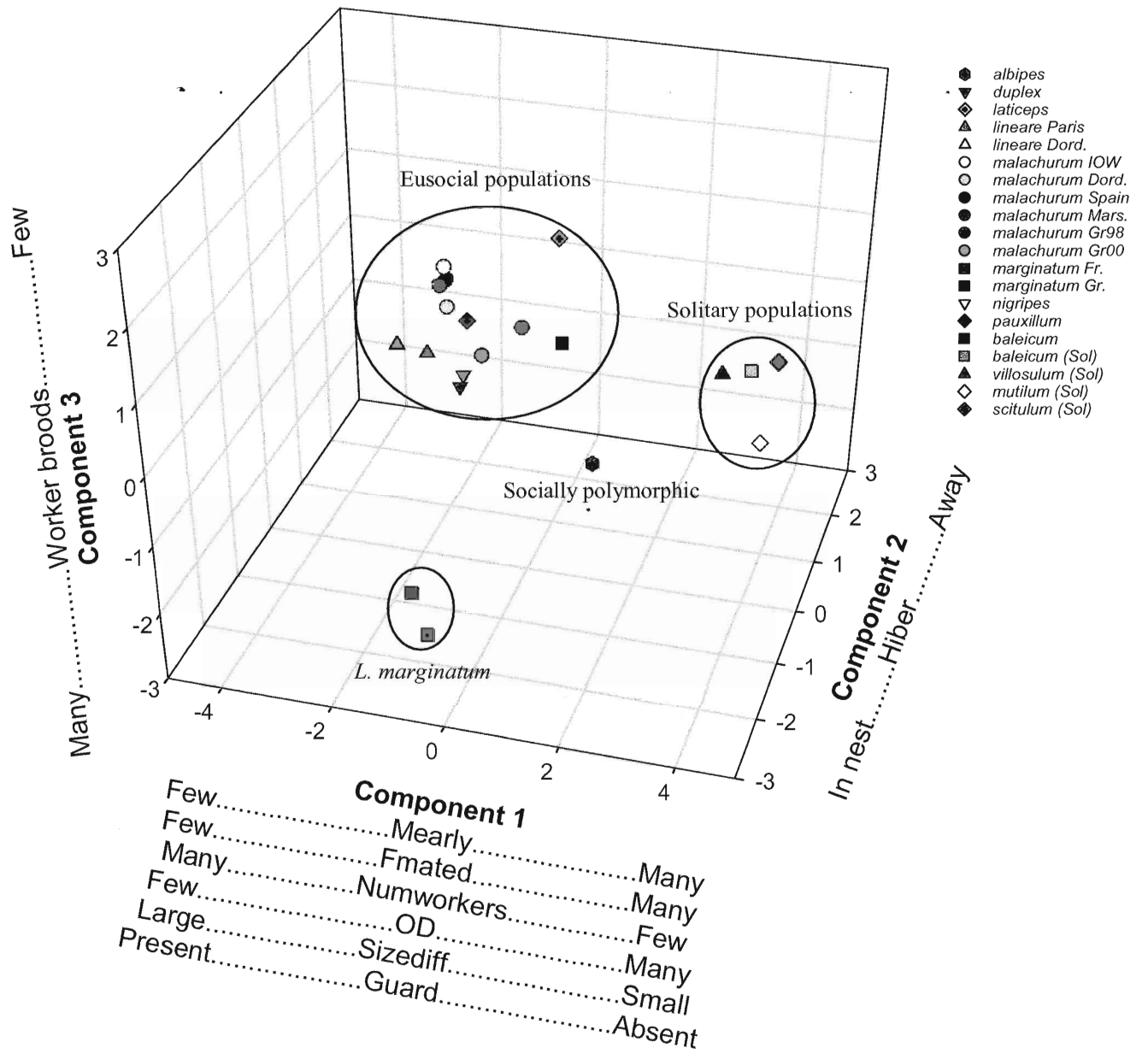

Figure 2.1: The positions of 14 eusocial, 2 socially polymorphic species and 3 solitary populations of *Evylaeus* and *Lasioglossum* with respect to the first three principal components, which explained 84% of the total variance. The expressions of the characteristics that had the highest loading factors are shown for each principal component. Mearly = % males in the early brood; Fmated = % of females mated; Numworkers = mean number of workers in the first brood; OD = % females with developed ovaries; Sizediff. = % Size difference between queens and workers; guard = nest guarding; Hiber = Overwintering locale of gynes and Worker broods = the number of worker broods.

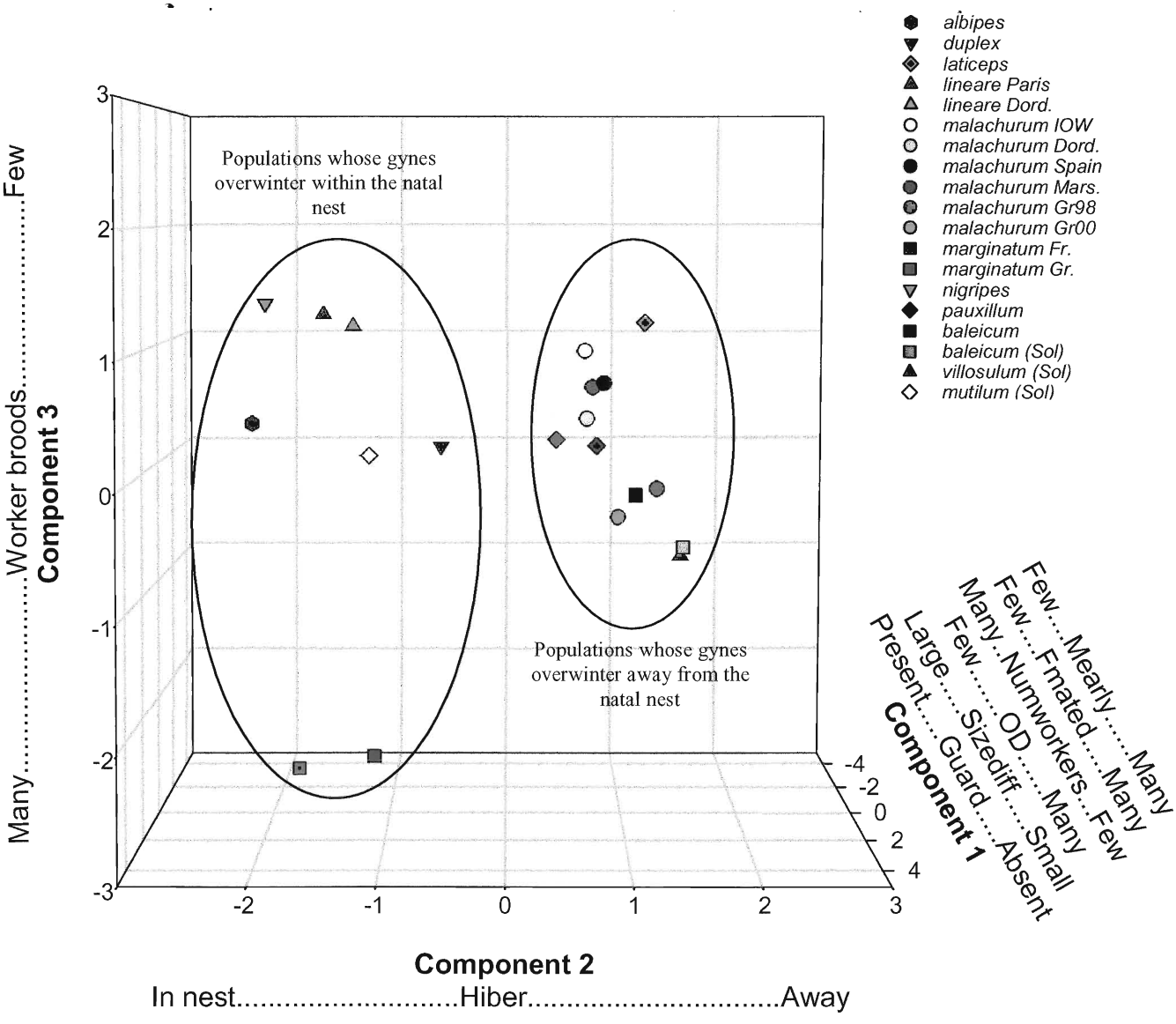

Figure 2.2: The positioning of the same 20 *Evylaeus* species and populations as in Fig. 2.1, but from the perspective of principal components two and three. Short forms the same as in Figure 2.1.

component one. The characteristics that are most significant to defining this component, and therefore are traits that separate eusocial from solitary populations are: the proportion of males in the early brood, the proportion of females mated and with developed ovaries, the number of first brood workers, caste size dimorphism and nest guarding. The *L. marginatum* populations are outliers away from the other eusocial species, driven primarily by component three. Figure 2.2 shows the positioning of the same *Evylaeus* species and populations as in Figure 2.1, but from the perspective of principal components two and three. A distinction can be observed between species or populations whose gynes overwinter within the natal nest from species or populations whose gynes overwinter away from the natal nest, based on PC2. *L. marginatum* is again separated from the rest of the *Evylaeus* group as a result of the number of worker broods on PC3.

Table 2.4 shows the results of the principal components analysis excluding the solitary and *L. marginatum* populations. The first three principal components explain 78% of the total variance and the eigenvalues drop below one after three components.

Only one characteristic, the proportion of males in the early brood, had a significant loading score (-0.84) on the first principal component, which explained 39% of the total variance. Six other characteristics had moderate loading scores (0.60-0.69) on this component. In association with stronger eusociality and a decrease in the proportion of males in the early brood, these were, haplometrotic nest founding, a decrease in the proportion of mated females, larger caste size differentials, gynes overwintering away from their natal nest and the presence of a guard at the nest entrance.

Principal components two and three explain 26% and 13% of the total variance, respectively. The mean number of first brood workers was the only significant trait (-0.70) on PC2 along with the overwintering locale of gynes having a moderate loading score (0.62). The proportion of workers with developed ovaries (0.82) was the only trait to significantly define PC3.

Table 2.4: Results of the principal components analysis for *Evylaeus*, excluding the solitary and *L. marginatum* populations. Loading factors scores of ≥0.70 were considered significant, and ≥0.60-0.69 were considered moderately significant, to defining a principal component.

	Loading factors		
Variable	**Factor 1**	**Factor 2**	**Factor 3**
Type of nest founding	-0.68908	-0.35565	0.38334
% Males in early brood	-0.84026	0.37702	-0.13176
% Females mated	-0.66722	0.50230	0.02203
% Size difference Q>W	0.65714	-0.56922	-0.15422
Overwintering locale	0.64230	0.62398	-0.14868
% Females with developed ovaries	0.28043	0.3301	0.82029
No. of worker broods	0.64175	0.43019	0.36753
Mean no. of 1st brood workers	0.47137	-0.70298	0.18060
Presence or absence of a guard	0.60746	0.53999	-0.30628
Eigenvalue	**3.5521**	**2.3518**	**1.1451**
Percent Explained	**39.5**	**26.1**	**12.7**
Cumulative Percent	**39.5**	**65.6**	**78.3**

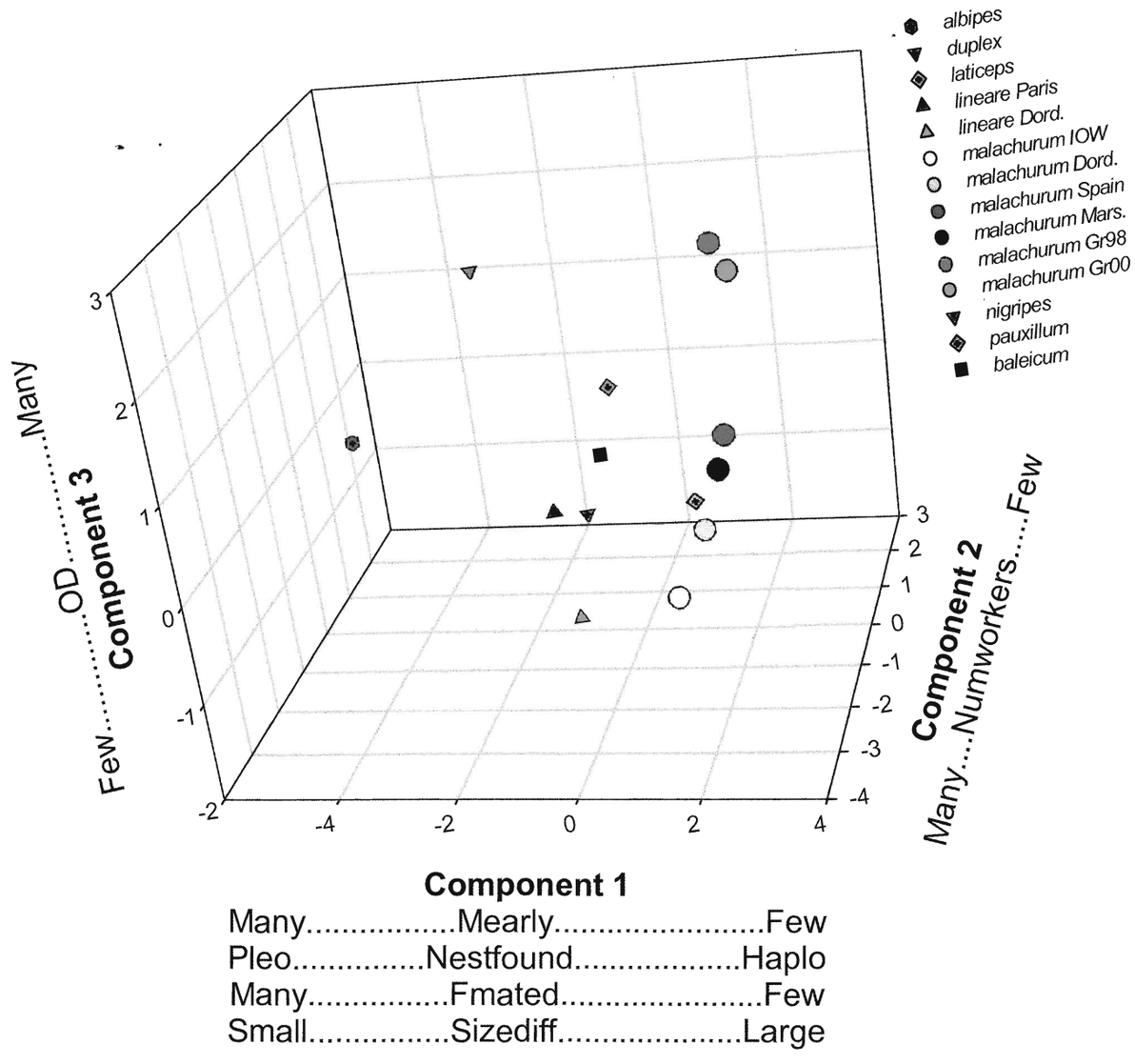

Figure 2.3: The positioning of 14 eusocial *Evylaeus* species and populations. Short forms the same as in Figure 2.1. The enlarged circles represent *L. malachurum* populations.

Discussion

L. malachurum exhibits intraspecific geographic variation in social behaviour. The two important traits that are associated with social variation are colony size and worker ovarian development (see Chapter One). This comparative study was performed in order to determine if factors associated with intraspecific variation in *L. malachurum* are the same as those that explain social level and social type in *Evylaeus* as a whole. Social level is used to describe populations or species that exhibit varying degrees of eusociality on a spectrum from weak to strong. Social type is defined as a species' or population's social organization, for example, solitary, eusocial or socially polymorphic. It must be pointed out that the following discussion refers primarily to *Evylaeus*, but two of the solitary populations included are *Lasioglossum* (*Lasioglossum*), and are discussed along with solitary *Evylaeus*.

Which behavioural traits are most closely associated with social variability in Evylaeus?

The traits that have the highest loading scores (e.g. ≥0.70) on principal component one and are most closely associated with stronger eusociality are: (1) a reduction in the proportion of males during the worker brood(s); (2) a reduction in the proportion of mated females; (3) an increase in the mean number of workers in the first brood; (4) a reduction in the proportion of females with developed ovaries; (5) an increase in size dimorphism between castes; and (6) nest guarding. Principal component one defines the differences among eusocial, solitary and socially polymorphic species, as indicated by a clear separation of the eusocial populations, which have low PC1 values, and the solitary populations, which have high PC1 values. The socially polymorphic *L. albipes* population falls in between. The *L. albipes* population (Plateaux-Quenu et al., 2000) used in the analysis was from the south-west of France (Dordogne and near Paris) and may represent a transitional population given that this species is polymorphic, but it is difficult to make conclusions because the data were obtained from a lab reared population.

Individual traits

Principal component one

The proportion of males in the early brood(s) is the trait with the highest loading score on principal component one, suggesting that this trait is the most important trait defining social type in *Evylaeus*. Solitary foundresses produce many males, whereas social foundresses produce few or no males early in the colony cycle. Solitary foundresses produce a 1:1 investment ratio of males to females because the reproductive value of sons and daughters is equal, since an equal sex ratio maximizes mating success for both sexes (Trivers and Hare 1976). Eusocial foundresses produce more females in the early brood because for every male produced, a worker is not, and workers can be more valuable than males to a eusocial foundress (at the beginning to mid part of the colony cycle) for maximizing her own reproductive success and the success of the colony.

The proportion of females mated is highly significant in defining principal component one, suggesting that worker reproduction is important to defining social type in *Evylaeus*. Solitary foundresses produce daughters that all mate, while social foundresses produce early brood daughters of which few mate. Solitary daughters must mate if they are to produce their own daughters the following year. In eusocial species, fewer workers are mated because workers that are mated may be more likely to reproduce for themselves than non-mated workers, and the degree of worker reproduction is inversely proportional to worker altruism, i.e. helping behaviour that may increase the queen's reproductive success. An increase in the proportion of mated females is correlated with an increase in the production of males early in the colony cycle. Therefore, *L. albipes* situated almost right in between the eusocial and solitary populations provides evidence that male production early in the colony cycle is probably an intermediate step required for the loss of eusociality because it increases the potential for workers to mate and discourages altruistic behaviour.

The proportion of workers with developed ovaries is one important trait defining social type in *Evylaeus* because it is significant in defining principal component one. In solitary species, all females must have well-developed ovaries in order to lay eggs. In social species, variation in the proportion of females with developed ovaries is observed, but queen reproduction (i.e. egg laying) usually exceeds that of her worker daughters. Variation in the proportion of workers with developed ovaries (i.e. potential egg layers) might be inversely proportional to worker altruism and associated with varying strengths of eusociality, as with the case of mated workers.

An increase in the mean number of first brood workers is significant in defining principal component one and therefore is associated with defining social type. Solitary species do not produce workers, while eusocial species produce workers in the first brood. In socially polymorphic species, the worker brood is facultatively expressed. It is omitted in the solitary populations and is present in the eusocial populations.

A large size difference between castes is closely associated with social type because this trait has the fourth highest loading score on principal component one. Solitary species do not exhibit size variation between castes because no workers are produced. Eusocial species can exhibit a range in size dimorphism between queens and workers. Large queens that produce small workers can control the degree of worker altruism by effectively preventing workers from reproducing and forcing them to forage, provision and/or guard. Queen manipulation of worker behaviour through physical aggression has been documented in sweat bee species such as *H. ligatus* (Richards and Packer 1996) and *L. zephyrum* (Kukuk and May 1991). The largest size variation in *Evylaeus* was observed in *L. lineare* in Dordogne, France (Packer and Knerer 1985). A size difference between castes of 21% was recorded, and only 0.5% of workers were mated and only 3.2% of workers had developed ovaries. In this case, queen manipulation is a viable hypothesis to account for reduced worker reproduction (Packer and Knerer 1985). In the socially polymorphic *L. albipes*,

queen-worker size dimorphism was only 5.6% and all workers in this laboratory- reared population were mated (Plateaux-Quenu et al., 2000). In this species, workers may be more successful at evading queen control, which might be required as an intermediate step in eusocial evolution. One exception is observed in *L. marginatum*, which is the only sweat bee for which no caste size dimorphism exists but in which workers are strongly altruistic (Michener 1974; Packer and Knerer 1985). Thus, queen manipulation by physical force is not a plausible hypothesis to explain worker altruism in this species.

In this study, nest guarding has the sixth highest loading score on principal component one. Solitary species do not have guards at their nest entrance (Sakagami and Munakata 1972; Plateaux-Quenu et al., 1989; Miyanaga et al., 1998; Miyanaga et al., 1999; Miyanaga et al., 2000; Cronin and Hirata, unpub.) because the foundress is the only occupant of the nest that must provision her developing brood, the critical period during the colony cycle. Many primitively eusocial species have a guard at the nest entrance (Michener 1974; Knerer 1992; Brothers et al., 2000). This individual may be the queen, especially during the first phase of worker foraging or, in many cases, one of the workers. The adaptation of a guard at the nest entrance may have been driven by increased parasite pressure, or by nest usurpation by conspecifics (Brothers et al., 2000). Increased colony size may facilitate nest guarding as seen in *L. pauxillum*, *L. malachurum* and *L. marginatum* in Greece and promote cooperation among colony members to ensure brood survival. Packer and Knerer (1985) suggested that a better measure of the efficiency of nest defense would be the mortality rates of the brood within nests and of whole colonies in order to show that brood survivorship is enhanced by the presence of a social structure. Unfortunately, relevant data are often not available.

Principal component two

The overwintering locale of gynes is the most (and only) significant trait that defines the second component. Although this trait is associated with defining variation among *Evylaeus*, no significant trend or pattern among the species is revealed (Fig. 2.2). This trait is not associated with social type or social level.

Principal component three

The number of worker broods has the highest loading score on component three and is the only significant trait on this component. The variation associated with component three distinguishes *L. marginatum* from all the other *Evylaeus* species. *L. marginatum* has a perennial colony cycle that includes five worker broods, one produced each year, before the reproductive brood (Michener 1974; Packer and Knerer 1985), while other *Evylaeus* produce a maximum of three worker broods in one year. An increasing number of worker broods, each with successively more workers, means those colonies can grow to be quite large because, as with the case in *L. marginatum*, a queen invests in a first brood of few workers that will in turn provision and forage for the next brood and so on, while the queen remains in the nest to lay eggs. Colony size, therefore, is thought to be an important factor that may influence the degree of worker altruism and the strength of eusociality because larger colonies may be indicative of increased cooperation and increased colony success. This is the case for *L. marginatum* in which colony size can be very large, and is correlated with low to no worker reproduction and highly altruistic workers.

On the other hand, if the colony size exceeds the queen's ability to control worker behaviour, it may provide opportunities for worker reproduction. For example, *L. malachurum* produces up to three worker broods and colony sizes can become quite large (Knerer 1992; Richards 2000; this study), but Brood 1 and/or Brood 2 workers later in the colony cycle may be involved in male production. But from a queen's point of view, by

producing many workers who help to produce many more reproductives than she could on her own, a proportion of male-producing workers may be a worthwhile trade-off (as long as she remains the primary reproductive).

The trait that was not important

The type of nest founding does not have significant or moderate loading scores (i.e. ≥0.60) on any component, suggesting that this trait is not associated with describing social type, or any variation, within *Evylaeus*. Most *Evylaeus* used in this study exhibit single-foundress nesting behaviour (18/25 species and populations), suggesting that the semisocial route to eusociality was not the one taken (Packer and Knerer 1985). In other words, multiple foundress associations are not required for the establishment or loss of eusociality.

Explanation for social variation in Evylaeus

The interpretation of the first three principal components suggests that a reduction in the production of males early in the colony cycle, a reduction in the proportion of mated females and females with developed ovaries, an increase in the number of first brood workers, an increase in the size difference between queens and workers, and increased nest defense are the critical differences between solitary and social species. These are all behaviours or characteristics that are under foundress control. In solitary species, a foundress produces a first brood of reproductives (i.e. males and future foundresses). Conversely, a eusocial foundress produces a first brood of workers to help her raise the reproductives. And in between, a socially polymorphic foundress produces either a first brood of reproductives or of workers. The implications of foundress control suggest that for facultatively eusocial species, queens can decide whether the nest will be social or solitary and therefore socially polymorphic nesting behaviour may represent an intermediate step in the loss or gain of eusocial nesting. For example, in Cape Breton, Nova Scotia,

Augochlorella striata is facultatively eusocial and populations contain both solitary and eusocial nests; this location represents the northern limits of its range (Packer 1990). The facultative expression of a worker caste was suggested to be dependent on ecological factors (Packer 1990). Consequently, *A. striata* foundresses can produce either a first brood of males and workers or a brood of males, workers and gynes, depending on which is the best strategy to maximize their reproductive success under the current ecological constraints.

In further support of foundress control among eusocial species, queen control through manipulation is a viable hypothesis for most *Evylaeus* because queens control demographic and other nesting factors. These include the sex ratio, the colony size (at least the number of workers), the size of the workers (by controlling the amount of food provisioned) and worker behaviour (by reducing their reproductive contributions and forcing them to guard and forage). This overall pattern is essentially what was revealed through Packer and Knerer's (1985) principal components analysis, and they suggested that parental manipulation has been an important factor in the evolution of social behaviour in *Evylaeus*.

Taking a closer look at the eusocial populations, by performing a principal components analysis excluding the solitary populations along with the *L. marginatum* populations, shows that only three traits are important for describing social level in *Evylaeus*. Moreover, these traits were distributed over the first three principal components, suggesting that different factors are important to defining social level than are required to describe social type in this subgenus. The proportion of males in the early brood is the only significant trait on PC1. The distribution of the populations suggest that the *L. malachurum* populations along with the *L. pauxillum* population are more strongly eusocial than the other eusocial *Evylaeus*, since fewer males are produced early in the colony cycle compared to the other *Evylaeus* used in the analysis. As discussed above, strongly eusocial foundresses produce more females than males in the early brood as they are more valuable to her for maximizing her reproductive success. The mean number of first brood workers is the only

significant trait on PC2 and does not appreciably describe social level among the eusocial *Evylaeus* (figure not shown). The proportion of females with developed ovaries is the only significant trait on PC3. The positioning of the *L. malachurum* populations clearly supports the hypothesis of a cline of decreasing eusociality based on an increasing proportion of workers with developed ovaries as populations are found more south (Fig. 2.3).

CONCLUSIONS

Altruistic behaviour is one of the most interesting evolutionary phenomena because it is a trait that promotes the reproductive success of others then progeny (Michener 1974; Crozier and Pamilo 1996). Altruism is best studied in the sweat bees, as they exhibit a social repertoire that includes a range of nesting behaviours, from solitary nesting to varying levels of eusociality. Furthermore, eusociality has evolved and reversed repeatedly in this group of bees. The subgenus *Evylaeus* is an excellent group for revealing factors important for the selection of altruism and eusociality because it contains species that exhibit solitary, socially polymorphic or varying levels of eusocial colony organizations. For example, *L. malachurum* is widely distributed in Europe and current evidence suggests a north-south cline of decreasing eusociality. The objectives of this thesis were to: (1) investigate intraspecific social variation in *L. malachurum* at a locale that is further south than any population studied thus far, (2) to test the hypothesis of a southerly cline of decreasing eusociality, and (3) to determine which colony, demographic, and ecological factors were most closely associated with defining intraspecific social variation in this species and social variation in the subgenus, *Evylaeus*, as a whole.

The field study conducted in May and June of 2000 provided a great deal of information about the colony phenology, colony demography and other nesting behaviours of *L. malachurum* in southern Greece. This information provided a basis for comparison of social level between *L. malachurum* in Greece and other European populations studied to date, and the ability to address the question of a north-south cline of decreasing eusociality. In Chapter One I concluded that, in Greece, *L. malachurum* is strongly eusocial because of a well established caste system of queens that monopolized reproduction and workers that were primarily altruistic. I also concluded that my results substantiate intraspecific social variation and support the hypothesis of a north-south cline of decreasing eusociality because

a higher proportion of workers potentially involved in male production was observed in Greece compared to more northerly populations.

One of the objectives of this thesis was to determine if factors associated with intraspecific geographic variation in *L. malachurum* are the same as those that explain social level and social type within *Evylaeus* as a whole. The comparative analysis performed on twenty solitary, eusocial and socially polymorphic *Evylaeus* and *Lasioglossum* populations, using principal components analysis, proves very useful for discerning which factors are most closely associated with defining social type in *Evylaeus.* A clear distinction between the solitary species, the eusocial species and the socially polymorphic *L. albipes* is evident and the traits that are most closely associated with eusociality are: (1) a reduction in the proportion of males produced during the worker brood(s); (2) a reduction in the proportion of mated females; (3) an increase in the mean number of workers in the first brood; (4) a reduction in the proportion of females with developed ovaries; (5) an increase in size dimorphism between queens and workers; and (5) nest guarding. Furthermore, the principal components analysis reveals that the two traits that are important for defining social variation (i.e. social level) within *L. malachurum*, colony size (i.e. the number of workers and number of worker broods) and worker ovarian development, are also factors that help to define social variation (i.e. social type) within the subgenus as a whole.

When comparing social level among only the eusocial species, (by excluding the solitary species along with the socially aberrant *L. marginatum* populations from the analysis), it is clear that the *L. malachurum* populations are separated by the proportion of workers with developed ovaries. The most northerly population, the Isle of Wight, U.K, has the least workers with developed ovaries and the most southerly Greek populations have the most, along with the other populations that line up in order of decreasing eusociality as the number of worker broods produced increases (except for the Spanish population). This

provides further evidence in support of the hypothesis that *L. malachurum* exhibits a north-south cline of decreasing eusociality across its geographic range.

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APPENDIX A

Table A1: Brood sizes of nests excavated at ANM and MALA1 based on live brood and parasites. All nests were from ANM, except 70 and 76, which were from MALA1. The numbers of parasites include *L. malachurum* larvae with parasites attached and parasite larvae that were the sole occupants of their brood cells.

Brood	Week	Nest	No. live brood	No. parasites	Total
2	**2**	70	20	0	20
		76	45	0	45
		386	23	10	33
		388	18	14	32
		390	34	3	37
		Subtotal	**140**	**27**	**167**
		Mean ± s.d.	**28.6 ± 10.6**		**33.4±2.8**
	4	382	13	4	17
		385	8	5	13
		Mean ± s.d.	**11 ± 2.8**	**9**	**30**
3	**5**	379	36	4	40
	6	376	56	0	56
		377	28	3	31
		378	60	1	61
		B1	45	0	45
		Subtotal	**189**	**4**	**193**
		Mean ± s.d.	**46.8 ± 13.7**		**48.3±13.3**
	7	426(P)	22	0	22
		427(P)	35	0	35
		Subtotal	**57**	**0**	**57**
		Mean ± s.d.	**28.5 ± 9.2**		**28.5±9.2**

(P) Partially excavated nests

Table A2: The proportion of active nests for weeks 1-7 at MALA1 based on nest surveys. The proportions of active nests were calculated based on the number of nests with foragers and or guards divided by the total number of nests each week.

	Week						
Observation	**1**	**2**	**3**	**4**	**5**	**6**	**7**
	No. nests						
Forager(s) and/or guard	31	52	4	16	9	5	2
Open	19	34	26	48	30	9	19
Closed	14	21	75	49	22	12	16
No recent digging	-	18	19	31	73	105	96
Total	64	125	124	144	134	131	133
% Active	**48.0**	**42**	**3.2**	**11.1**	**6.7**	**3.8**	**1.5**

Table A3: Mean head width (mm) ± standard deviation of nest occupants from excavated nests from Broods 1-3. Numbers in brackets represent the number of individuals with measured head widths.

Brood	Nest	Queens	Brood 1 workers	Brood 2 workers	Brood 3 workers	Gynes	Males
2	**70**	2.45	2.18±0.05 (3)	-	-	-	-
	76	-	2.16+0.08 (5)	-	-	-	-
	386	2.51	2.23±0.05 (5)	2.20±0.12 (16)	-	-	-
	388	2.48	2.21±0.04 (3)	2.13±0.14 (10)	-	-	-
	390	2.39	2.29±0.05 (6)	2.13±0.11 (11)	-	-	-
	382	2.42	2.20±0.04 (2)	2.20±0.04 (14)	-	-	-
	385	2.23	2.10±0.07 (7)	2.10±0.06 (13)	-	-	-
	Mean±SD	**2.41±0.10 (6)**	**2.20±0.06 (31)**	**2.15±0.05 (64)**	-	-	-
3	**379**	-	-	2.23±0.04 (14)$^{+}$	2.33±0.00 (1)	2.43±0.02 (2)	1.95±0.06 (5)
	376	2.51	-	2.18±0.04 (10)$^{+}$	2.22±0.15 (2)	2.48±0.00 (1)	1.88±0.04 (8)
	377	2.51	-	2.22±0.06 (12)$^{+}$	2.23±0.0 (1)	2.47±0.04 (3)	1.82±0.08 (4)
	378	2.57±0.04 (2)	-	2.19±0.06 (11)$^{+}$	2.27±0.09 (3)	2.45±0.02 (9)	1.93±0.08 (8)
	A1 $^{(p)}$	2.48	-	2.20±0.00 (1)$^{+}$	-	2.46±0.02 (3)	1.95±0.00 (1)
	B1	2.45	-	-	2.29±0.08 (4)	2.47±0.01 (5)	1.92±0.12 (6)
	426 $^{(p)}$	2.48	-	2.28±0.07 (5)$^{+}$	2.36±0.00 (1)	2.49±0.01 (5)	2.01±0.04 (12)
	427 $^{(p)}$	2.39	-	2.20±0.04 (2)$^{+}$	2.25±0.08 (3)	2.53±0.07 (12)	2.00±0.10 (11)
	Mean±SD	**2.50±0.06 (8)**	-	**2.21±0.03** (55)	**2.27±0.08 (15)**	**2.48±0.04 (40)**	**1.94±0.07 (55)**

(P) Partially excavated nests $^{+}$May be Brood 1 or Brood 2 worker